4th Concertation meeting on
Photonics-Enabled Applications

10-11 September 2009, Athens, Greece

Optical sensing in lab-on-a-chip by femtosecond laser written waveguides

Nicola Bellini, Andrea Crespi, Rebeca Martinez Vazquez,
Yu Gu, Roberto Osellame, Giulio Cerullo

Dipartimento di Fisica – Politecnico di Milano
HIBISCUS PARTNERS

Hybrid Integrated BIophotonic Sensors
Created by Ultrafast laser System

- Leibniz University of Hannover (Germany)
- HIGHQLASER GmbH (Austria)
- Politecnico di Milano (Italy)
- University of Twente (Netherlands)
- LIONIX BV (Netherlands)
- University of Hull (UK)
- Zebra Bioscience (Netherlands)
OPTICAL DETECTION IN LOC

- Strong development of LAB-ON-A-CHIP (LOC): miniaturized bio-chemical laboratory in a single “mm-scale” chip

  - Type of analysis (e.g. DNA analysis, drug discovery, medical diagnostic)
  - Type of fluidic components (e.g. channels, reservoirs, valves, pumps)
  - Type of developed technologies (e.g. wet etching, dry etching, soft lithography)

In-situ OPTICAL DETECTION systems:
bulk optical elements (e.g. mirrors, lenses, microscope objectives)

- Miniaturized LOC with massive benchtop instrument for detection
- Strong limitation in system portability
- Suffer mechanical vibrations and drifts
INTEGRATION OF OPTICAL SENSING IN LAB-ON-CHIPS:
e.g. microchip capillary electrophoresis (MCE)

MOTIVATION & GOAL

- Bio-molecules are identified on the basis of the arrival time at the detection point

Critical and challenging issue
NONLINEAR ABSORPTION of FEMTOSECOND LASER PULSES inside a glass substrate can provide structural modifications (i.e. refractive index variation for waveguides or pre-etching structure for microchannels).

Devices can be fabricated by translation of the sample.

**Advantages:**
- can be applied to DIFFERENT materials
- NO need for photolithography
- FLEXIBLE technique for fast prototyping of devices
- 3D CAPABILITY of device writing
- µ-processing portfolio on fused silica

**Advantages for integrated optical detection in LOCs:**
- single-step post-process on commercial LOCs
- integrated, compact and portable on-chip detection system
TWO DIFFERENT APPROACHES

• **1st approach:**
  Laser Induced Fluorescence (LIF) detection
  
  **Labelled biomolecules:**
  - high sensitivity
  - low limit of detection

• **2nd approach:**
  Refractive index detection by Mach-Zehnder Interferometers (MZIs)
  
  **Label-free biomolecules:**
  - no alteration of molecule functions
  - reliable quantitative analysis
ON CHIP DETECTION IN CAPILLARY ELECTROPHORESIS LAB-ON-A-CHIP

1st approach: LASER INDUCED FLUORESCENCE DETECTION
Waveguide diameter of 10 µm and astigmatic beam shaping for circular cross-section.
Maximum refractive index change of about $5 \times 10^{-4}$ → single mode in the whole visible range

Propagation losses (PL) by \textit{cut-back} method at 543 nm:

Near-field of the guided modes:

<table>
<thead>
<tr>
<th>Waveguide Material</th>
<th>Losses in the green</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiON</td>
<td>1 dB/cm</td>
<td>Opt.Lett.26,716 (2001)</td>
</tr>
</tbody>
</table>

R. Osellame et al. APL 90, 231118 (2007)
WAVEGUIDE INTEGRATION IN A COMMERCIAL BIOCHIP

In-coupling
WAVEGUIDE INTEGRATION IN A COMMERCIAL BIOCHIP

Laser-Induced-Fluorescence (LIF)

- microchannel filled with rhodamine 6G
- laser at 543 nm coupled into the waveguide
- microscope image through a cut-off filter at 560 nm

very good spatial accuracy

10 µm

50 µm

Optical waveguide

Blow-up
Detection system

- Notch filter 532 nm
- Interferential filter 560 nm
- PMT-Hamamatsu H6240

0.48 NA
300 µm core radius (fused silica core/polymeric cladding)

Fiber pigtailing to the fs-laser written waveguide

FLUORESCENCE DETECTION SCHEME

Fluorescence collection

Laser light coupling
LIMIT OF DETECTION (LOD)
Very good linearity between concentration and detected fluorescence

Limit of detection ~ 40 pM ⇒ state of the art value for on chip approach

R. Martinez Vazquez et al., Lab Chip 9, 91-96 (2009).
Front Cover of the leading journal in the field

January 2009 issue
DYE SEPARATION BY ELECTROPHORESIS

- CCD imaging of laser induced fluorescence by a fs-laser written waveguide
- Sample is a highly concentrated solution of two different dyes (Rhodamine 6G and Rhodamine B)

OLIGONUCLEOTIDE ELECTROPHORESIS

- Fiber collection set-up with PMT to assess the sensitivity
- 23-mer Cy3-labelled oligonucleotide

- Excellent temporal resolution
- Very high sensitivity down to 1nM

2nd approach:

REFRACTIVE INDEX DETECTION BY MACH-ZEHNDER INTERFEROMETERS
REFRACTIVE INDEX SENSING IN MCE

Interferometer in the Mach-Zehnder configuration (MZI)

- Need for spatial resolution $\Rightarrow$
  interferometer **ORTHOGONAL** to the separation channel

- Channel width of only $L = 50 \mu m$ $\Rightarrow$
  evanescent field sensing too weak $\Rightarrow$
  one arm of the interferometer is **CROSSING** the channel

Theoretical phase shift

$$\lambda L_n = \Delta \Lambda$$

Sensitivity

$$\frac{\Delta \lambda}{\Lambda} / \Delta n = -\frac{L}{\lambda}$$
**WAVEGUIDE WRITING: HIGH REP RATE**

- **Yb:KYW laser**
  - Mode-locking and cavity-dumping
  - $f_{\text{rep}} = 400 \text{ kHz} \div 1 \text{ MHz}$

- **SHG stage**
  - Increases the energy absorption and the waveguide quality

- **Translation stages**
  - Aerotech Fiberglide air-bearing 3 axes
  - Accuracy 100 nm

**Parameters**
- $\lambda = 1030 \text{ nm}$
- $\tau = 350 \text{ fs}$
- $E_{\text{pulse}} = 1 \mu J$
- $\lambda = 515 \text{ nm}$
**WAVEGUIDE WRITING: HIGH REP RATE**

- **Fabrication parameters:**
  - 1MHz, 350 fs
  - 90 nJ @ 515 nm
  - 100 µm/s
  - 0.6 NA

- **Waveguide mode:**
  - $\lambda = 1550 \text{ nm}$

Estimated refractive index change $\Delta n = 5 \times 10^{-3}$
CURVED WAVEGUIDES: Y-SPLITTERS

- **S-bent** waveguides with different radii
- Symmetric **Y-splitters** by double scan with overlapping common arm

![Bending Losses Graph]

Bending Loss < 1 dB/cm for R > 20 mm

- very reproducible splitting ratio 52-48%±1%

![Imaging Diagram]

R = 30 mm

2.4 mm

100 μm

100 μm
MACH-ZEHNDER CHARACTERIZATION

- Unbalanced MZI to measure the properties of the device:

- Transmission spectrum using tunable laser (1460-1570 nm):

\[ T = \frac{1}{2} \left[ 1 + \cos \left( \frac{2\pi}{\lambda_0} n_0 \Delta s \right) \right] \]

- \( \Delta s_{th} \approx \Delta s_{meas} \Rightarrow \)
  - good control of uniformity in the two arms

- HIGH fringe VISIBILITY (> 10 dB) \( \Rightarrow \)
  - symmetric splitting ratio at any \( \lambda \)
  - same propagation losses in the two arms

L=17mm; h=50\,\mu m; R=30mm; \Delta s=56\,\mu m
• In the ORTHOGONAL CROSSING configuration:
  one arm is crossing the microchannel,
  the other arm has to be below or above it

\[
T = \frac{1}{2} \left\{ 1 + \cos \left[ \frac{2\pi}{\lambda} \left( n_0 \Delta s - (n_{ch} - n_0) L \right) \right] \right\}
\]
REFRACTIVE INDEX DETECTION

- Different concentration of aqueous GLUCOSE-D solution
- Acquisition of the entire SPECTRUM in a finite time
- Calculation of the PHASE of the Fourier transform

**Static measure**

- Very good linearity: phase shift VS concentration
- Very good sensitivity ~ $3 \times 10^{-5}$ Refractive Index Unit (RIU)

A. Crespi et al., Lab Chip, submitted (2009)
CONCLUSIONS

- Integration of 3D optical devices fabricated by femtosecond laser technology on commercial LOCs has been achieved.

- On-chip sensing in capillary electrophoresis microchip has been investigated in:
  - laser induced fluorescence
  - refractive index measurements

- Both static and dynamic measurements have been tested with good success.

- Femtosecond laser micromachining is now a mature technology that is ready to go out and compete with other ones. Optofluidics is probably the optimal playground to express the potentials of this technology.