Micron-sized laser particles for massively multiplexed cellular labelling and tracking

Nicola Martino*, Sheldon J. J. Kwok*, Andreas C. Liapis, Sarah Forward, Sun-Joo Jang, Seok-Hyun Yun
Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts, United States;
Department of Dermatology, Harvard Medical School, Boston, Massachusetts, United States;
Massachusetts Institute of Technology, Cambridge, Massachusetts, United States.
** Equal contribution*
Author e-mail address: syun@hms.harvard.edu

Abstract: Laser particles are a promising technology for massively multiplexed cellular labelling and tracking. We demonstrate the fabrication of optical barcodes with four hundred distinct spectral channels, their uptake in cells and stability in biological environments.
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The critical role of cellular heterogeneity has been identified in a number of biological processes, from stem cell biology to cancer progression [1-2]. Fluorescence microscopy is commonly used to study cellular behavior *in vitro* and *in vivo*. However, conventional fluorescent probes such as organic dyes and proteins have relatively broad emission bandwidths (~100 nm), which limits unambiguous imaging of up to only four cell types due to spectral overlap. To fully capture the diversity of cellular behaviors, which can involve hundreds of distinct cell types in a tumor, probes with much narrower emission bandwidths are needed.

Compared with the incoherent emission of fluorescent probes, lasers have a coherent output with dramatically narrower bandwidth (easily less than 1 nm). We recently proposed that laser particles that are free standing and compatible with biological systems can serve as optical probes in biomedical imaging, cytometry, and assays [3]. In this conference, we presented our initial work on the fabrication of microdisk laser particles from a III-V compound semiconductor, $In_{0.53}Al_{0.17}Ga_{0.38}As$ emitting in the range of 1250-1320 nm.

Here, we present our latest results on the generation of laser particles with emission spanning from 1200 to 1600 nm by using multiple semiconductor wafers based on InAlGaAs and InGaAsP with varying compositions. Each laser particle is about 1 µm in radius and coated with biocompatible materials, and upon optical pumping at 1060 nm, emits a single laser mode with a linewidth of less than 1 nm. We demonstrate that our laser particles are readily internalized by cells and that their emission remains stable over the course of repeated measurements, demonstrating their suitability as highly-multiplexed optical barcodes with roughly 400 spectral channels.

Microdisks cavities were fabricated from these wafers with a standard photolithographic process using SU-8 as negative resist, followed by reactive ion etching (RIE). After releasing the disks from the InP substrate using a selective etchant (HCl), they were suspended in an ethanol solution and coated with a ≈ 100 nm thick layer of silica. This coating helps decouple the resonating cavities from changes in external refractive index, improves water solubility, and confers biocompatibility for cellular internalization [4].

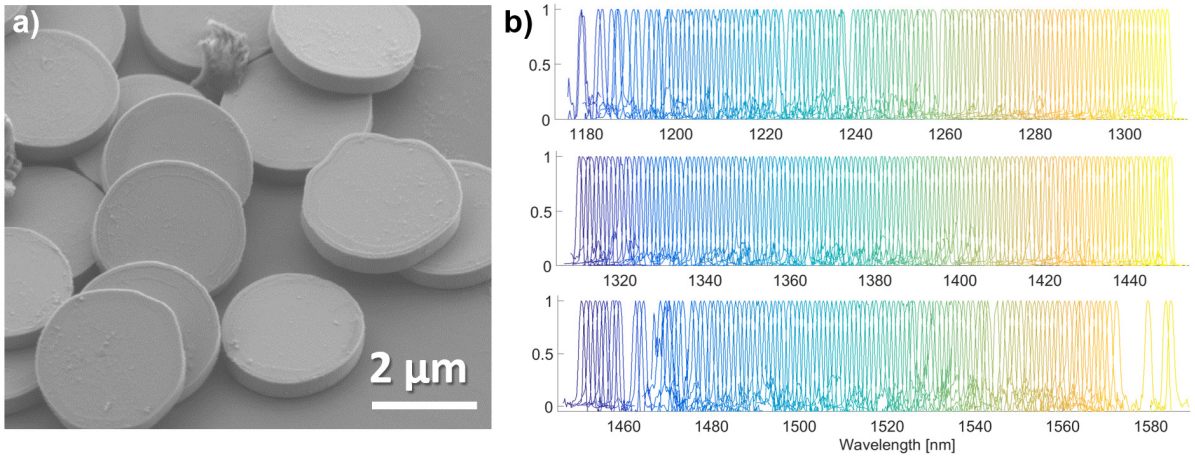

Figure 1: a) SEM image of several microdisk cavities released from their original substrate and deposited on a silicon chip; b) emission spectra of $n = 390$ disks (normalized) with ≈ 1 nm spacing covering the wavelength range from 1180 nm to 1580 nm

For optical characterization, we embedded our laser particles in a 3D matrix made of Matrigel, a laminin and collagen based preparation that simulates the extracellular environment of biological tissues. We measured the emission spectra of several hundreds of these laser particles by pumping them with a 3 ns / 500 kHz pulsed laser centered around 1060 nm. Figure 1b shows the spectra of $n = 390$ selected laser particles, with distinct emission peaks spanning 1180 to 1580 nm in approximately 1 nm steps. The measured cavities usually have threshold pump energies in the range between 10 and 20 pJ, with best-case values down to 5 pJ (data not shown).

Laser particles in aqueous solution were added to *in-vitro* cultures of Madin-Darby Canine Kidney (MDCK) cells to test their biocompatibility. After 24 h – 48 h of incubation, most of the disks are internalized by the cells via a non-specific micropinocytosis process [5]. The panels in Figure 2a show two snapshots taken at different times of MDCK cells which have internalized several laser particles; most cells have at least one microdisk. The cells remain viable and keep proliferating, as can be seen by the presence of mitotic cells (see panel for $t = 6\ h$). Figure 2b shows a higher resolution view of a laser particle inside a MDCK cell, in which a fluorescent dye is incorporated into the silica coating.

To track laser particles in *in-vitro* cultures and track them as the cells move, we combined the spectroscopy setup with a laser-scanning confocal microscope coupled to a SWIR spectrometer. The pump laser was scanned over the sample with a pixel dwell-time of 200 µs and a resolution of ≈ 1 µm per pixel. The peak wavelength extracted from the acquired spectra was used to identify and track individual disks over 50 sequential measurements (~ 3 hours total observation time). Figure 2c reports the distribution of wavelengths, measured at each time point, for some of the disks tracked during the experiment; The plots demonstrate that the peak emission of the laser cavities remains constant, within less than 1 nm.

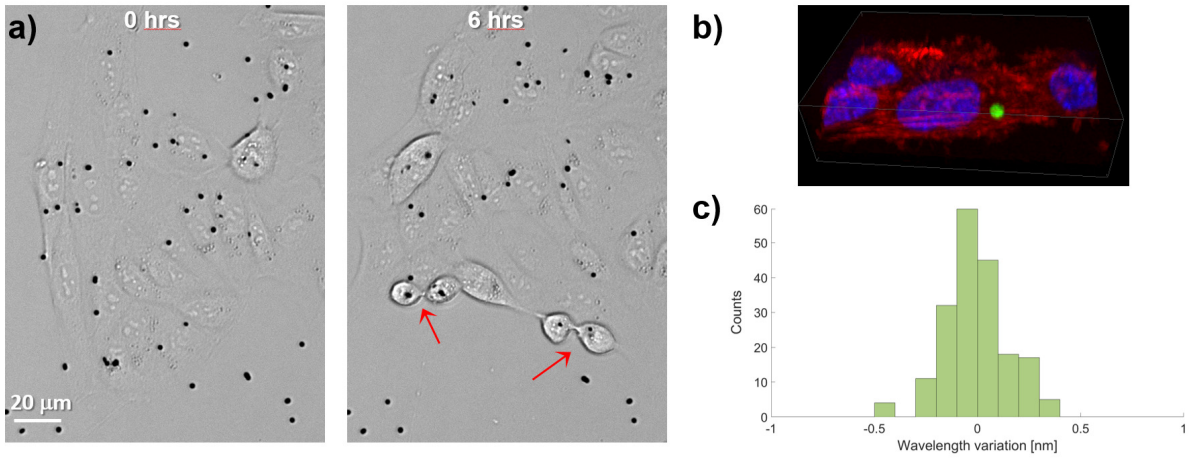

Figure 2: a) laser particles internalized by MDCK cells at two different times after seeding; b) 3D confocal fluorescent image of a laser particle (green: laser particle, blue: nuclei, red: actin filaments); c) distribution of peak wavelength variations for $n = 10$ disks in cells over 50 consecutive measurements

Using microdisk lasers, we have demonstrated the fabrication of several hundreds of distinct optical barcodes, and verified that their emission wavelength remains stable over repeated measurements inside cells. We envision using them to track the behavior of large heterogeneous populations of cells in 3-dimensional cultures or in mice *in vivo*.

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