Microlaser-based contractility sensing in single cardiomyocytes and whole hearts

Marcel Schubert[1,*], Lewis Woolfson[1], Isla RM Barnard[1], Andrew Morton[1], Becky Casement[1], Gavin B Robertson[2], Gareth B Miles[3], Samantha J Pitt[2], Carl S Tucker[4], Malte C Gather[1]

1 School of Physics and Astronomy, University of St Andrews
2 School of Medicine, University of St Andrews
3 School of Psychology & Neuroscience, University of St Andrews
4 The Queen's Medical Research Institute, University of Edinburgh
** ms293@st-andrews.ac.uk*

Abstract: Microscopic whispering gallery mode lasers detect minute changes in cellular refractive index inside individual cardiac cells and in live zebrafish. We show that these signals encode cardiac contractility that can be used for intravital sensing.
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1. Introduction

Characterizing the contraction of single cells in the beating heart is the ultimate challenge for understanding heart diseases and for advancing cardiac regeneration therapies. However, currently available techniques and probes lack speed and sensitivity as well as single cell specificity.

Here, we introduce a spectroscopic technique to extract transient contraction profiles of beating cardiomyocytes based on intracellular microlasers. The sensitivity of microlaser emission to local changes in refractive index allows us to gain quantitative results that can be linked to the protein density of the contractile myofibrils. We further explore unique advantages of microlasers like long-term tracking of individual cells and real-time monitoring of drug administration. To explore the translational perspective of our work, we performed experiments in isolated primary cardiomyocytes and live zebrafish. Our results will stimulate novel translational approaches by accessing the functional properties of transplanted cells and engineered cardiac tissue *in vitro* and *in vivo* reliably and non-invasively.

2. Results

Whispering gallery mode (WGM) microresonators belong to the most advanced biosensors and have reached sensitivities down to the single molecule and protein level [1,2], but their potential for intracellular and *in vivo* sensing remain largely unexplored. Only recently, microlasers were proposed as novel optical tags to uniquely discriminate hundreds of thousands of cells and to track cells over extended periods in time [3–6]. Here, we show that spherical microlasers with a size of about 15 µm can be internalized by neonatal mouse cardiomyocytes (CMs). A typical WGM spectrum is shown in **Fig. 1**. During contraction, the modes reveal pulse-shaped perturbations in lasing wavelength which are synchronized across all modes and coincident with the spontaneous contractions of the CMs.

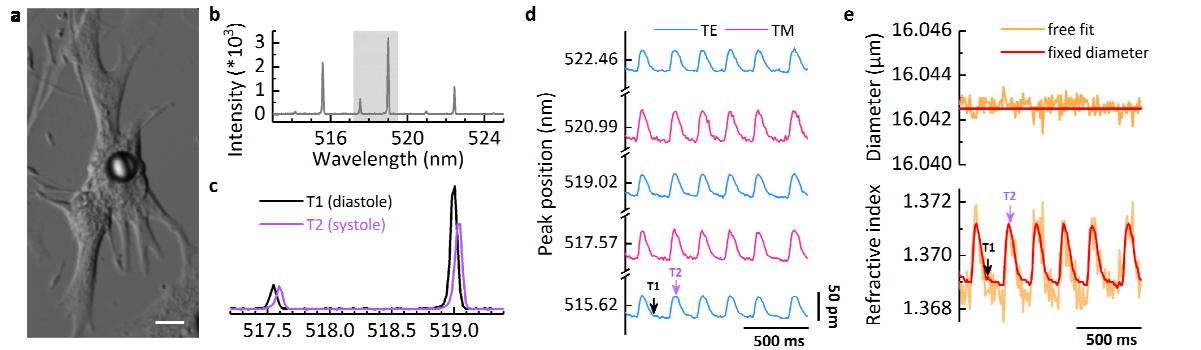

Fig. 1. a, DIC microscopy of a group of neonatal CMs and an intracellular microlaser. Scale bar, 15 µm. **b**, Whispering gallery mode lasing spectrum of a 16 µm polystyrene microlaser located inside a neonatal cardiomyocyte. **c**, Zoom-in to a TM- and TE-mode pair at 2 different time points in the contraction cycle of the cell. **d**, Mode position under continuous lasing reveals periodic red shifts in the laser mode position. **e**, Calculated diameter (top) of the microlaser (orange) and time-averaged diameter (red). External refractive index (n_{ext}, bottom) calculated with unrestricted microlaser size (orange) and by applying the fixed mean diameter of the microlaser (red).

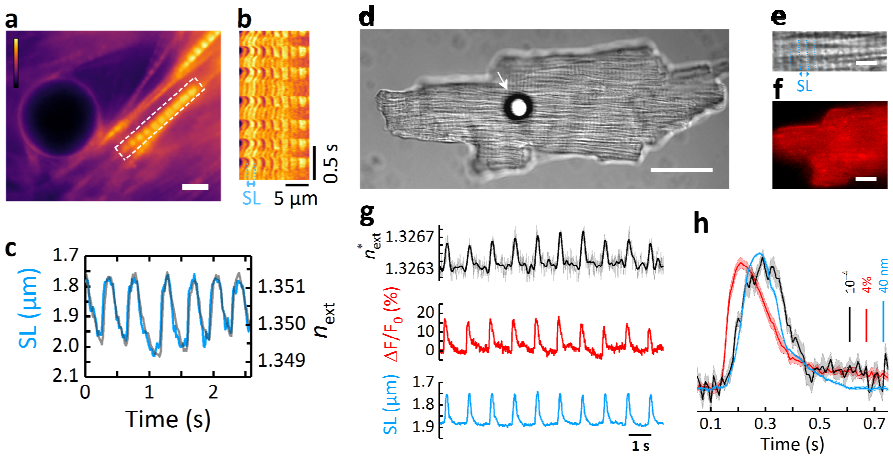

Fig. 2. Microlasers monitor cellular contractility **a**, Fluorescence microscopy of neonatal mouse CMs with labelled myofibrils (SiR-actin). The intracellular microlaser is visible as dark circular object. **b**, Kymograph of a single myofibril used to extract sarcomere length (SL). **c**, Simultaneously acquired temporal profiles of sarcomere length (SL, blue, left axis, extracted from fluorescence profiles of the myofibrils highlighted by the white rectangles in **a**) and cellular refractive index n_{ext} (grey, right axis, extracted from microlaser spectra). **d**, Extracellular microlaser (white arrow) on top of an adult CM. Scale bar, 30 µm. **e**, Magnified view showing highly organized myofibrils (sarcomere repeat units indicated by dashed blue lines). Scale bar, 4 µm. **f**, Optical calcium sensing with the fluorescent calcium indicator (XRhod1). **g**, Temporal profiles of the refractive index (black), calcium signal (red) and sarcomere length (blue) of an electrically paced cell. **h**, Averaged profiles of the traces shown in g.

By using optical modelling and after applying statistical analysis, the diameter of the microlaser and the refractive index inside the CM were determined with high precision (**Fig. 1e**). We find that contractions consistently led to an increase of cellular refractive index and that measurements can be performed continuously over more than 10 minutes without affecting the characteristics of the cells. To identify the origin of the contraction-induced increase in refractive index, correlative contractility measurements were performed in neonatal CMs by staining sarcomeric f-actin fibres (**Fig. 2a-c**). These experiments reveal that the cellular refractive index is directly correlated to the sarcomere length. Thus, transient signals of the microlasers provide a direct measure of CM contractility.

Microlaser-based contractility measurements can also be combined with multimodal sensing as we demonstrate by simultaneously characterizing contractility and calcium signaling in isolated mouse CMs (**Fig. 2d-h**). We further injected microlasers into the heart of zebrafish embryos and were able to detect locally resolved contractility profiles under *in vivo* conditions.

3. Discussion

Multifunctional probes which monitor the long-term integration of injected stem cells or engineered cardiac tissue are urgently needed to improve cardiac regenerative therapies. Microlaser-based contractility measurements can monitor the contractile properties of individual cells during various developmental stages without the need for staining or genetic alteration. Being a purely spectroscopic technique, our method is expected to be more resilient to scattering than imaging-based methods since scattering in biological tissue is elastic and hence does not alter spectroscopic information. By providing single cell specificity, long-term tracking, reduced sensitivity to scattering, and with potential reductions in resonator size [7], microlasers introduce new possibilities for translational approaches that extend well beyond current microscopy-based techniques, offer reduced complexity and impose fewer experimental restrictions.

4. References

1. F. Vollmer and S. Arnold, "Whispering-gallery-mode biosensing: label-free detection down to single molecules," Nat. Methods **5**(7), 591–596 (2008).
2. E. Kim, M. D. Baaske, I. Schuldes, P. S. Wilsch, and F. Vollmer, "Label-free optical detection of single enzyme-reactant reactions and associated conformational changes," Sci. Adv. **3**(3), e1603044 (2017).
3. M. Schubert, A. Steude, P. Liehm, N. M. Kronenberg, M. Karl, E. C. Campbell, S. J. Powis, and M. C. Gather, "Lasing within Live Cells Containing Intracellular Optical Microresonators for Barcode-Type Cell Tagging and Tracking," Nano Lett. **15**(8), 5647–5652 (2015).
4. M. Schubert, K. Volckaert, M. Karl, A. Morton, P. Liehm, G. B. Miles, S. J. Powis, and M. C. Gather, "Lasing in Live Mitotic and Non-Phagocytic Cells by Efficient Delivery of Microresonators," Sci. Rep. **7**, 40877 (2017).
5. M. Humar and S. H. Yun, "Intracellular microlasers," Nat. Photonics **9**(9), 572–576 (2015).
6. M. Humar and S. H. Yun, "Whispering-gallery-mode emission from biological luminescent protein microcavity assemblies," Optica **4**(2), 222 (2017).
7. A. H. Fikouras, M. Schubert, M. Karl, J. D. Kumar, S. J. Powis, A. di Falco, and M. C. Gather, "Non-obstructive intracellular nanolasers," Nat. Commun. DOI: 10.1038/s41467-018-07248-0 (2018).