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Current Opinion in Biomedical Engineering

Expansion mini-microscopy: An enabling alternative in point-of-care diagnostics

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Diagnostics play a significant role in health care. In the developing world and low-resource regions the utility for point-of-care (POC) diagnostics becomes even greater. This need has long been recognized, and diagnostic technology has seen tremendous progress with the development of portable instrumentation such as miniature imagers featuring low complexity and cost. However, such inexpensive devices have not been able to achieve a resolution sufficient for POC detection of pathogens at very small scales, such as single-cell parasites, bacteria, fungi, and viruses. To this end, expansion microscopy (ExM) is a recently developed technique that, by physically expanding preserved biological specimens through a chemical process, enables super-resolution imaging on conventional microscopes and improves imaging resolution of a given microscope without the need to modify the existing microscope hardware. Here we review recent advances in ExM and portable imagers, respectively, and discuss the rational combination of the two technologies, that we term expansion mini-microscopy (ExMM). In ExMM, the physical expansion of a biological sample followed by imaging on a mini-microscope achieves a resolution as high as that attainable by conventional high-end microscopes imaging non-expanded samples, at significant reduction in cost. We believe that this newly developed ExMM technique is likely to find widespread applications in POC diagnostics in resource-limited and remote regions by expanded-scale imaging of biological specimens that are otherwise not resolvable using low-cost imagers.

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Current Opinion in Biomedical Engineering 2017, **1**:45–53

This review comes from a themed issue on **Future of BME**

Edited by **George Truskey**

Received 12 January 2017, revised 24 February 2017, accepted 8 March 2017

http://dx.doi.org/10.1016/j.cobme.2017.03.001

Keywords
Expansion microscopy, Miniature imager, Portable, Low-cost, Point-of-care.

Introduction

Diagnostics play a significant role worldwide by providing proper and timely healthcare to patients [1]. The role of diagnostics and in particular, the need for point-of-care (POC) diagnostics, is even more critical in the developing world and low-resource regions [1–3]. This has been recognized for long, and diagnostic technology has seen tremendous progress with the development of, in particular, imaging-based strategies.

Although conventional optical microscopy provides high-end capacities in terms of resolution, often times their bulky sizes, expense, and need for alignment and adjustment cannot satisfy the need of portable observations. Recent advances in POC diagnostics have further challenged existing microscopy techniques where *in situ* and cost-effective detection is required in addition to sufficient sensitivity and resolution. For example, compact image sensors are widely applicable to POC analysis at mammalian cell level [4–9]. The mini-microscopy has several key advantages over conventional

microscopy in undeveloped regions [10], including: *i*) simplicity, light weight, and compactness: a mini-microscope can be easily constructed with simple design, making the system compact and portable, well suited for POC diagnostics; *ii*) cost-effectiveness: the main components of a mini-microscope include off-the-shelf items such as a light-emitting diode (LED) and an image sensor, making it affordable in POC diagnostics. Nevertheless, while the mini-microscopes are widely applicable in portable POC diagnosis at the scale of mammalian cells, it remains challenging for their utility in detecting disease-causing pathogens (*e.g.* parasites, bacteria, viruses, and fungi) due to the limited resolution of their equipped low-end optics and/or sensors.

Expansion microscopy (ExM) is a recently developed technique that enables super-resolution imaging (*e.g.* below the diffraction limit) on conventional microscopes and improves imaging resolution of a given microscope without the need to modify the existing microscope hardware. ExM relies on a very simple principle, *i.e.* embedding a biological sample in a matrix of swellable polymer, which chemically anchors fluorescent labels already pre-applied to the specimen, and then physically expands the specimen by swelling the specimen-matrix composite to ~4–5 times in linear dimension [11]. Variations of ExM have been further developed recently to permit the simple, powerful anchoring and separation of proteins and nucleic acids of biological samples [12,13].

As such, the rational combination of ExM and mini-microscopy may potentially push forward a new initiative in POC diagnostics, which we term expansion mini-microscopy (ExMM) [14]. We have thus demonstrated that even a low-cost mini-microscope could image biological samples (both mammalian and bacterial cells) at unprecedentedly high resolution comparable to conventional microscopy without expansion, indicating that the integration of the original version of ExM with a mini-microscope through our ExMM technology can result in resolution that rivals that of a conventional microscope with a hundred-to-thousand-fold reduction in the cost. Here in this Opinion Article, we review recent advances in ExM and portable imagers, respectively, and discuss the preliminary findings of their combinatory form, the ExMM. We finally conclude with future perspectives on the potential application of ExMM in POC diagnostics.

Expansion microscopy (ExM)

Conventional imaging of specimens with high spatial precision requires expensive equipment, because precise magnification with a minimum of aberration and a maximum of sensitivity requires high-end lenses and cameras, as well as precision alignment. We recently developed a new strategy depending primarily on chemistry to do the magnification – physically instead of optically [11]. We discovered that, by synthesizing a swellable polyelectrolyte hydrogel network directly within a specimen of interest, and subsequently dialyzing the sample in a medium of lower osmolarity (*i.e.* pure water), it could be physically expanded (Figure 1a–c). Specifically, we used sodium polyacrylate as the hydrogel material, which when synthesized in high salt is compact, but when the salt is diluted undergoes swelling due to electrostatic repulsion (Figure 1ai–ii). By staining a sample with a trifunctional label comprised of an antibody, a polymer-linking group, and a fluorophore, we were able to anchor the fluorophore to the hydrogel network (Figure 1d–f); by enzymatically digesting the endogenous structure, we were able to render the sample mechanically homogeneous. Water, then, swells the sample (Figure 1b *versus* 1c). Embedding preserved biological specimens in hydrogels for microscopy imaging purposes has a long history, dating back to 1995 [15], but expansion microscopy uses a specific property of hydrogels – in particular polyelectrolyte gels – namely the massive swelling of such hydrogel upon exposure to pure water, to physically move apart components of a biological specimen, so that nanoscale structures can be resolved.

Since the sample preparation procedures involve enzymatic homogenization of the mechanical characteristics of the tissue-polymer composite, this technology design enables isotropic and uniform expansion to occur [11]. Comparing images pre-*versus* post-expansion, using conventional (structured illumination-based) super-resolution microscopes, in fixed cultured HEK cells as well as in mouse brain slices, we confirmed that this expansion process was isotropic [11], and thus a ~4.5-fold expansion enabled an effective resolution of 300 nm (the diffraction limit of the lens used) divided by 4.5, or approximately 60 nm, approaching that attainable with classical super-resolution microcopy methods – but without requiring the hardware [16,17]. ExM has been utilized in super-resolution imaging of a variety of biological specimens using conventional optical microscopy, including for example, cultured mammalian cells [11], brain tissues [11], and cancerous tissues [18]. As shown in Figure 1, g and h, a Thy1-YFP-H mouse cortex slice was stained with antibodies against yellow fluorescent protein (YFP, green), as well as the pre- and post-synaptic scaffolding proteins Bassoon (blue) and Homer1 (red). The post-ExM image (Figure 1h) showed clear demarcations between the Bassoon/Homer1 signals while in the pre-ExM image (Figure 1g) the staining formed overlapping spots at each synapse due to blur by diffraction.

Further development of ExM

ExM is a chemistry that is easily customized for new applications. The original ExM method was unable to retain native proteins in the hydrogel and used specially designed reagents not widely available [11]. We subsequently developed a variant of ExM, named protein-