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Imaging Dynabeads with the Scanning Electron Microscope

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Ligand-coated Dynabeads (Invitrogen, CA) have many applications within life sciences, biotech, and healthcare for isolating specific subcellular material. The ligand can be any molecule (e.g. an antibody or antigen) with an affinity for the desired target. The superparamagnetic nature of the Dynabeads allows the beads in solution to be gently manipulated with magnets. Imaging subcellular material on Dynabeads with a scanning electron microscope (SEM) would take advantage of the specificity and separation of the isolation using Dynabeads and produce images at the micro- and nano-meter level.

Dynabeads are themselves small (a few micrometers in diameter) and are kept in solution. To view them and their contents with the SEM the beads must be removed from solution, dried, and mounted on SEM pegs. Rather than pulling the beads onto filters by suction, a common method for separating solids from solution, we decided to use magnets to collect and mount the beads for viewing. We tried two collecting methods. In the first method, we dropped the Dynabead solution directly onto a magnet using a pipette. A drop was maintained during fixation and an alcohol series while the beads adhered to the magnet. In the second method, we dropped the Dynabead solution onto a piece of millipore filter (Nuclepore, CA) which lied on top of a magnet. The filter with 0.2 micron diameter pores remained wet and on the magnet during fixation and the alcohol series. The beads were pulled to the filter by their attraction to the underlying magnet and covalently bound to the millipore filter during fixation (4% buffered glutaraldehyde or 1% buffered osmium). We first dried the beads on the magnets using a critical-point dryer, but found we lost too many beads. We then switched to drying with hexamethyldisilazane (HMDS; Ted Pella, CA) for both collecting methods. Besides preventing substantial bead loss, the use of HMDS allowed better tracking of the bead side of the filter. After drying, the beads were coated with gold.

We obtained good resolution of the beads down to the nano range using a LEO 1550 VP SEM (Figs. 1 and 2). This was true even with the beads positioned on magnets (Figure 1). A magnet did not overly interfere with the electromagnetic lens of the SEM or the scattered electrons coming off the specimen. Subcellular material on the beads was easily observed (Figs. 1 and 2).

In this experimental exercise we used the superparamagnetic nature of the Dynabeads to manipulate them during SEM processing. We then imaged the Dynabeads to see if subcellular material that is isolated using the beads can be structurally characterized directly on the beads. We imaged subcellular material isolated using Dynabeads quickly and easily with either of the two collecting methods and were limited only to the preservation methods for the material and the resolution of the electron microscope.

This work was done at the Wadsworth Center's EM Core Facility.

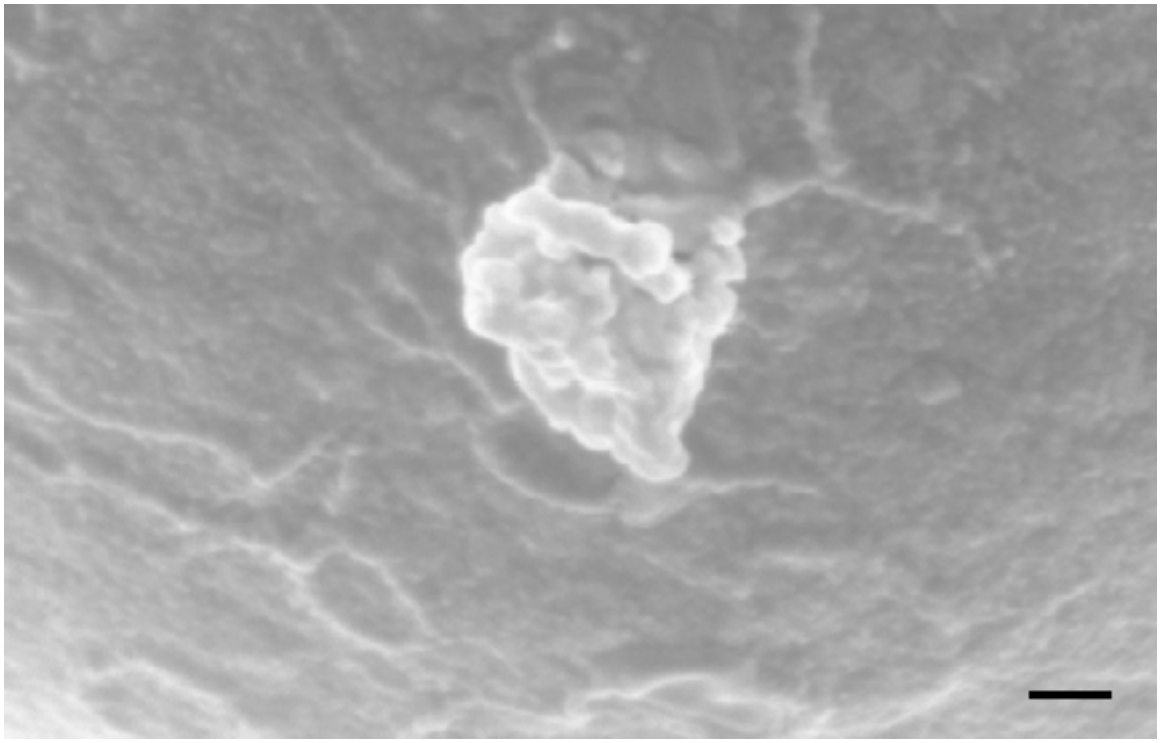

Fig. 1. Subcellular material attached to a 5.0-micron-diameter Dynabead on a magnet. Bar equals 200 nm.

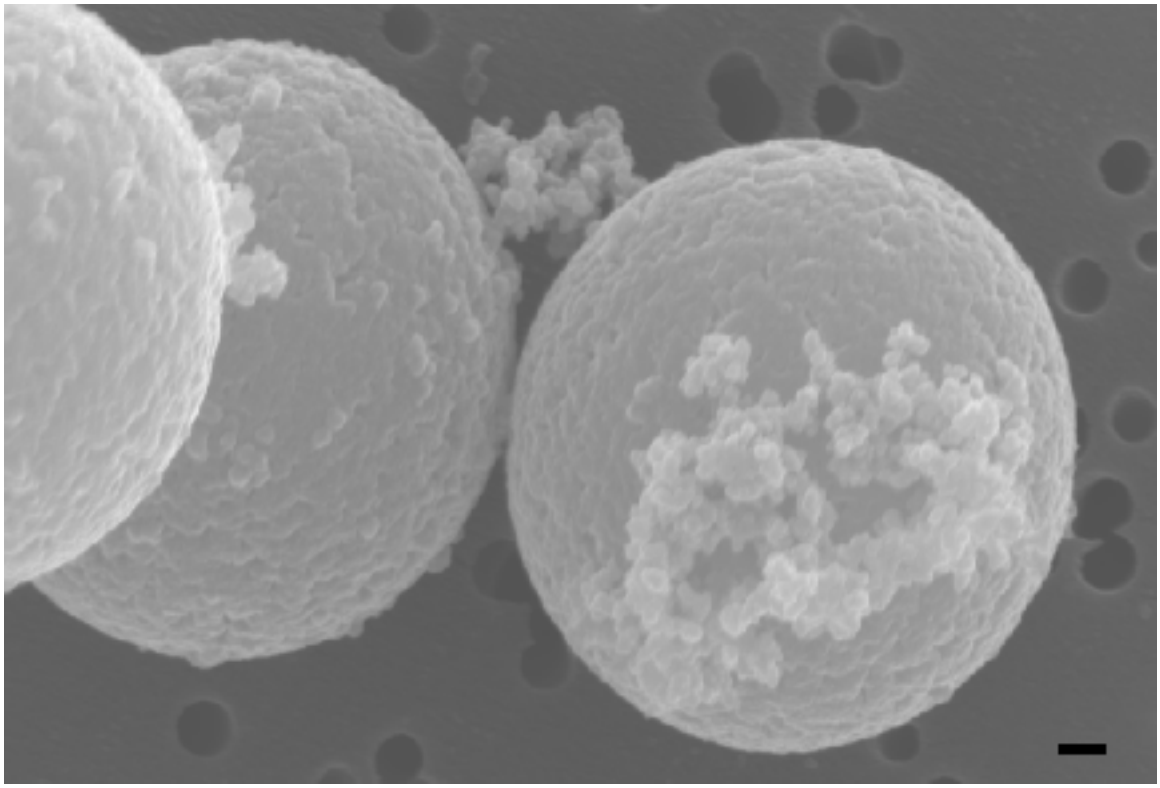

Fig. 2. Subcellular material attached to 2.8-micron-diameter Dynabeads on a millipore filter. Bar equals 200 nm.

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