Fixation and preparation of pellets from monolayer cell cultures

- Drain off the medium in which the cells have grown. There is no need to wash with cold buffer before applying fixative unless this is absolutely required in the cell preparation protocol.

- Add fixative and incubate for the required fixation time. e.g. 30 minutes in 0.5% glutaraldehyde in 0.2M PIPES, HEPES or cacodylate buffer pH 7.2.

- If necessary wash and store in PBS at 4 degrees C. Cells may be sent through the post at this stage by topping up the flask with buffer or fixative as appropriate.

- Drain off the buffer/fix and add 1ml of fixative (using fixative prevents the cells sticking to plastic centrifuge tubes).

- Scrape the cells into the fixative with a rubber scraper or cell scraper (quantitative studies in our laboratory have shown that scraping does not cause significant disruption to cell structures; J Lucocq unpublished results)

- Break up any sheets of cells by successive aspiration into a 1ml plastic pipette tip (if necessary control using phase contrast that the cell sheets have been broken-up)

- Centrifuge for at least 15 minutes at 10,000xg in fixative (note that the total fixation time is the fixation on the dish plus the centrifugation time.