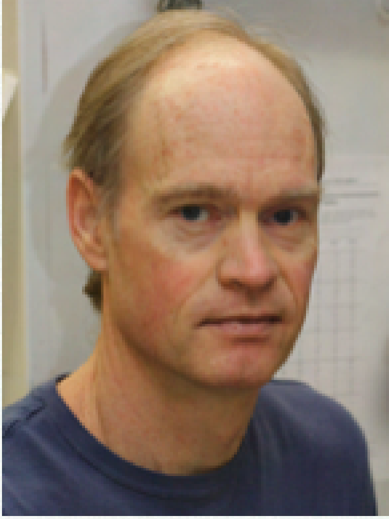
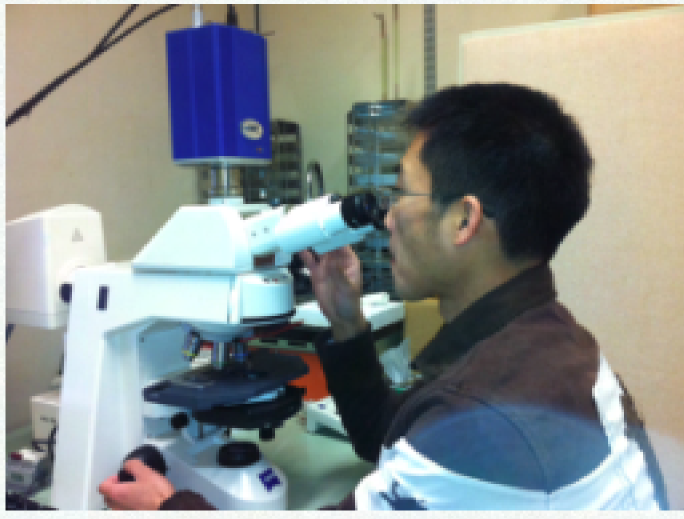
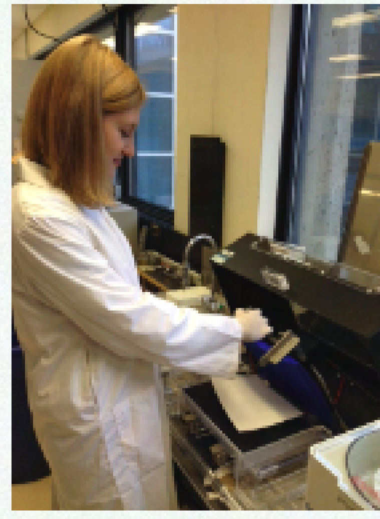
Lab Spotlight - Dr. David Stuart

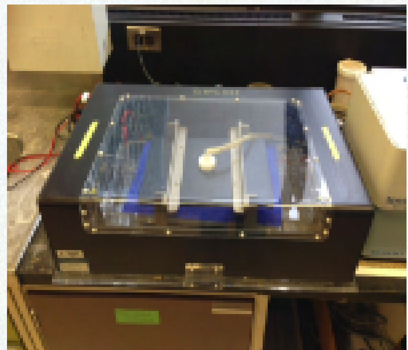
The Stuart lab in the department of biochemistry is housed on the fifth floor of the Medical Sciences Building and has been operating since 1998. The lab group has varied in size over the years and currently includes: Postdoctoral fellows, Dr. Bonnie McNeil, and Dr. Anagha Krishnan, MSc students, Yuze Xu and Brianna Greenwood will join us in September. Biochemistry undergraduate students, Lauren Graham and Kha Bui have just completed their Bioch 499 projects with us. During the summer of 2017 we will host undergraduate students Allan Yarahamady (U of A biochemistry) and Winston Gamache (Queens University biochemistry). The lab regularly hosts summer students, Bioch 498, Bioch 499, Bioch 398, WISEST and now Bioch 299 students.







The most active area of research in the lab is currently metabolic engineering and synthetic biology. Synthetic DNA is used to "rewire" metabolic pathways in yeast, cyanobacteria and *E. coli* to produce new products. Efforts are focused on engineering microbial cells to develop sustainable processes for the conversion of cellulosic waste material into fuels, replacements for petroleum-based chemicals, pharmaceuticals and nutraceuticals. We have introduced entirely new biosynthetic pathways into yeast to produce the biofuels butanol and isobutanol; engineered cells to produce hexadecanol and other long chain alcohols that can replace palm oil derived compounds; constructed a surfactant producing yeast and a microbial strain that converts pentose sugars from straw and sawdust into the low calorie sweetener erythritol. Recently, a probiotic yeast was constructed that secretes anti-inflammatory molecules that can control Inflammatory Bowel Syndrome and Crohn's Disease. Metabolic engineering also makes use of novel genetic screening techniques and directed evolution to improve the ability of microbial strains to produce the desired products. Some of the organisms we engineer do not have well developed genetics and we apply genome-engineering strategies using RED/ET recombination and CRISPR/Cas9 to introduce new genes or delete genes that encode enzymes that act in competing reactions with the pathway we are interested in. We have extensive experience with analytical techniques using mass-spectrometry (MS), gas chromatography (GC), high performance liquid chromatography (HPLC), and high throughput screening. Additionally, The fields of synthetic biology and metabolic engineering offer us enormous potential to not only construct microbial strains that meet our needs but to understand metabolism and biosynthetic pathways in greater detail than was previously possible.



A second area of active research is focused on investigating fundamental mechanisms that regulate cell growth, division, metabolism and differentiation. One of our favorite model systems for this has been the budding yeast *Saccharomyces cerevisae*. This organism is amenable to sophisticated genetic analysis and its genome can be readily modified to allow the deletion, insertion or mutation of genes. Typical cell cycle investigations include northern blotting or qPCR to monitor mRNA abundance, western blotting to monitor protein abundance and modification state, immunoprecipitation to monitor protein-protein interactions and protein kinase activities. We perform protein phosphorylation analysis through a mixture of mass-spectrometry, *in vivo* labeling with ^{32}P and 2-dimensional phosphopeptide mapping with a Thin Layer Chromatography / Electrophoresis system. We use our fluorescence microscope to observe chromosome dynamics, nuclear division and for localization of proteins within cells.

The lab uses a centrifugal elutriation centrifuge to isolate unperturbed populations of cells from any phase of the cell cycle. These can then be used for biochemical or cytological analysis. This unusual instrument allows the separation of cells based upon their size and shape allowing us to capture the smallest cells in a population, which correspond to the newborn G1-phase cells. This has allowed us to investigate the problem of cell size control and identify the means by which cell populations maintain their size homeostasis. This centrifuge has also been used to isolate specific cell types from mixed populations of mammalian cells for example we have isolated rat spermatagonial cells in various stages of meiosis from testicular homogenates.

Much of the labs work is directed to investigating the mechanisms by which cyclins and CDKs drive the cell cycle and how gene expression is controlled. Our work revealed how the cyclin Clb5 plays a unique role in driving DNA replication during meiosis and how its activity is regulated by the CDK inhibitor Sic1. Recently lab members combined yeast two-hybrid screening with immunoprecipitation, chromatin immunoprecipitation and site-directed mutagenesis to investigate the protein-protein interactions that allow histone deacetylase activity to repress meiosis-specific genes during mitotic growth. These investigations have clarified how the genes required for meiosis and meiotic recombination can be held silent when cells are actively growing and rapidly activated when receiving the signal to initiate meiosis. We will be continuing to dissect this process and investigate the factors that control the on/off switches that regulate meiosis-specific gene expression.

For more information about Dr. Stuart and his research, click HERE