**A. Instrument Location and Type**

* *Instrument Location:* The proposed instrument, an ImageStreamX, will be located at the research core facility in the Albro-Falconer-Manley Science Center at Spelman College in Atlanta, GA.
* *Instrument Code*: MRI-21 (microscopes) and MRI-24 (spectrometers). ImageStreamX is state-of-the-art image flow cytometer that combines the features of a flow cytometer and a fluorescence microscope.

# B. Research Activities to be Enabled

The requested ImageStreamX will enable four Spelman research groups to study cell population dynamics with combined morphometric and photometric features and advance their research to new levels. Moreover, it will enable many minority undergraduates to conduct research using state-of-the-art statistical imaging technology (Table 1).

**Table 1. Overview of the research activities**

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|  |  | Related Previous Results | | Anticipated ImageStreamX usage per year | |
| Groups (Sections) | Research activities enabled | # of publications [References] | UG involvement | Usage | UGresearchers |
| Qin (B.1) | Population dynamics and natural variation study on cellular aging of *Saccharomyces cerevisiae*. | 7 [1-7] , 1 in review,3 in preparation | ~25 UG trained, 5 UG co-authorships, 6 UG national, and 2 international meeting presentations | ~40% | ~3 |
| Maloney (B.2) | Function of glycosphingolipids in cell proliferation and apoptosis. | 11 [8-18] | ~30 UG trained, 5 UG co-authorship; 4 UG national meeting presentations | ~15% | ~2 |
| Jackson (B.3) | Dynamic behavior of androgen receptor in cell proliferation. | 4[20-23] | ~35 UG trained, ~ 5 national meeting presentations | ~15% | ~2 |
| Ibeanusi/Qin (B.4) | Population dynamics of bacterial cells in responses to metal ions stress. | 6 [24-29] | ~20 UG trained | ~15% | ~2 |
| Other students (B.5, D1, D2 ) | UG independent studies, projects in BIO487, ES435, BIO328, BIO233, & CHE313L. |  |  | ~15% | ~2 independent studies, and 13 groups for course projects |

Note: UG indicates undergraduates, and most of them are expected to be female African Americans.

## B.1 Population dynamics and natural variation study on cellular aging of *Saccharomyces* *cerevisiae*. (Qin)

### *Background on yeast aging*

Aging is a fundamental question in biology [30-32]. The aging of cells that undergo asymmetric divisions likely arose early in the evolution of both prokaryotes and eukaryotes [33-35]. As a unicellular organism, the budding yeast *Saccharomyces cerevisiae* has proven to be a good model system for studying the mechanisms of cellular aging [36]. Many key features of cellular aging were first discovered in yeast before they were established in metazoan cells [35, 37-40]. The life span of yeast can be measured in two ways: replicative and chronological life spans. Replicative life span (RLS) is the number of cell cycles that individual mother cells produce before they senesce and cease to divide [36, 41, 42]. The actual number of daughters produced by a cohort of mother cells is determined by microdissection. Chronological life span (CLS) indicates how long cells can survive without dividing in stationary phase [43, 44]. The number of surviving cells in a population is assessed over time by quantifying colony-forming units. Both replicative aging and chronological aging are defined based on the concept of cell cycle: RLS is a measurement of cell cycles that a single mother cell can accomplish, and CLS is the capability of cells to reenter the cell cycle from a non-dividing state.

A large body of experimental data in yeast suggests complex mechanisms of aging. In a large scale screen, deletion of 90 genes were found to extend chronological life span in BY laboratory strains, and only 16 of them are TOR related [45]. The remaining genes have functions associated with iron homeostasis, cell wall organization and biogenesis, transport, and many have unknown functions [45]. Deletion of 300 genes can shorten chronological life span [45]. In another screen of replicative life span, 20% of the gene deletions were found to shorten replicative life span, whereas 10 out 564 genes significantly extend replicative life span [51]. Six of them are implicated in the TOR pathway, four others are a ubiquitin protease, an isocitrate dehyodrogenase, and two proteins with unknown functions. In collaboration with Jeff Townsend at Yale University, we compared gene expressional profiles of short and long-lived segregants of awild yeast isolate [7], and found 15 genes with consistent differential expression levels between the long- and the short-lived progeny, including genes involved in gene silencing, stress response, and mitochondrial function.

Mitochondria play a important role in yeast cell aging [52]. Calorie restriction (CR) can extend both RLS and CLS in yeast. Several recent studies have suggested that CR reduces superoxide anions by elevating H2O2 levels, although details vary in different models [53, 54, 55-57]. SIR2 and TOR are found to be interconnected through MSN2/4 [58], and appear to both act through ROS signaling pathways to extend life span [55, 56, 59] , but conflicting evidence also exists [57]. In addition, Gottschling and colleagues reported that mitochondria dysfunction and non-respiratory function is a major determinant of nuclear genome stability [60]. Overall, although conflicting details remain unresolved, mitochondrial dysfunction and imbalanced ROS levels are considered as key factors of yeast aging [60].

The role of cell morphology in mitotic asymmetry and yeast aging is newly discovered topic [61]. As a single cell organism, it is important for mother yeast cells to prevent aging factors, such as damaged proteins, from passing on to new-born daughter cells. Surprisingly, this mitotic asymmetry can be sufficiently achieved by the slow diffusion of large aggregates, geometry of the mother and daughter cells, and the narrowness of the passage between them [61]. This observation is consistent with the morphological changes of yeast cells during replicative aging. During microdissection analysis of replicative aging, it can be seen that young yeast cells are generally smaller in size with elliptical shapes. Old yeast cells become larger, round in shape, and often have membrane blebbing (irregular bulges) [42, 62]. Mitotic asymmetry between mother and daughter cells breaks down around the time when most cells lose viability in the population. This leads to much higher levels of genomic instability in the daughter cells [63, 64]. The morphological changes coincide with the slow-down of cell division during replicative aging. Old mother cells become larger and take longer to divide. Daughter cells from young mother cells are usually much smaller than their mother cells, but daughter cells from old mother cells can often be similar in size to their mothers [62]. Similar break-down of mitotic asymmetry was also observed by us for chronological aging of yeast cells [5]. Our evidence suggest that the role of cell morphology in aging warrants detailed studies.

Age structure is important for the understanding of population dynamics and evolution [19]. Budding of each yeast cell will leave a scar on its cell wall, and these bud scars can be used to estimate the replicative age of cells [65-69]. A recent study using confocal fluorescence microscopy showed that yeast cells with more than 6 scars disappear rapidly in late log phase, perhaps through apoptosis [66]. The molecular mechanism by which this occurs remains poorly understood; primarilybecause this study was done in a single haploid S288c laboratory strain background in part because of the tedious process [66]. This technical challenge can be effectively addressed by the requested ImageStreamX, and we propose to quantify age structures in yeast natural isolates and deletion strains.

In addition to age structure, yeast cells in a population often exist in different physiological states. For instance, yeast cells growing in a log-phase are expected to exist in different phases of cell cycle: G1, S, G2, and M phases. The fraction of cells in G2/M phases is generally small, and is hard to be quantified by microscopic methods. Another example is yeast cells in stationary phase that consist both dividing cells and non-dividing cells (likely G0) [70]. Non-dividing yeast cells generated by starvation can also exist in several distinct states [71] and can cycle through high and low respiratory states [72-74]. Like age structure, these kinds of population heterogeneity can be effectively studied by ImageStreamX.

Despite the complexity of cellular aging due to both genic and non-genic factors, there are surprisingly robust statistical characteristics of aging at the cell population level in yeast, first reported in quantitative details by us [3]. Both replicative and chronological aging display the sigmoid shape of survival curves, which imply the exponential increase of mortality rate during the dying off phase. The exponential increase of mortality rate, also known as the Gompertz model [75], is a universal characteristic of aging in eukaryotic organisms, including yeast, worms, fruit flies, mice, and humans [3, 30, 76, 77]. The Gompertz model contains two key parameters: one parameter that describes the acceleration of mortality increase with time, termed the Gompertz parameter (*G*), and another parameter that describes the initial mortality rate (*R0*). The Gompertz parameter can be viewed as the rate of aging, and the *R0* is a proxy of the maximal life span potential. We found a negative linear correlation between G and ln(*R0*) in yeast [3]. This negative linear correlation was first reported in humans [78], known as the Strehler-Mildvan correlation, and has been extensively studied. This kind of universality is one of the motivations for mathematical modeling of cellular aging by PI Qin.

### *Preliminary results of yeast aging assay using conventional flow cytometer and its limitation.*

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| C:\Documents and Settings\hqin\My Documents\Dropbox\MRI.dropbox\sandboxMRI\figures\A3.071410\SangerA3-PI-viability\Slide1.TIF |
| Figure 1. Propidium iodide (PrI) staining can be used to measure chronological lifespan in *S. cerevisiae*. PrI-negative cells are considered as viable cells. Small inserts are histograms of log-transformed PrI signals. When cells become older, the PrI peak shift to the right, indicating more cell become PrI-positive. This experiment was done using a BD FACSCanto II at the FHCRC. |

Dr Qin has designed a protocol to measure CLS of the budding yeast using conventional flow cytometer at the Fred Hutchinson Cancer Research Center in the summer of 2010 and at the Princeton University in the summer of 2011. Briefly, aliquots of yeast cells in either depleted media or water are taken periodically, sonicated for 2 seconds, and are stained by propidium idodide (PrI). PrI is a fluorescent dye that enters cells with damaged membranes, and can be used as a marker for certain types of cell death [66, 79-81]. PrI negative cells are considered to be viable cells, and PrI positive cells are considered to be dead ones (Figure 1). The fractions of live and dead cells are modeled by t-mixture models with Box-Cox transformation or log-transformation, and are estimated by expectation maximization procedure using the R package flowClust [82, 83].

There are at least two major shortcomings of the PrI-based viability assay that can be greatly improved by the requested image flow cytometer. (1) PrI can stain nucleic DNA, mitochondrial DNA, and cytosolic RNA. This background signals vary substantially in ~70 yeast natural isolates that we measured. ImageStreamX can separate the more condensed nucleic DNA signals from the more diffused signals of mitochondria DNA or cytosolic RNA. (2) Some dead yeast cells are PrI-negative, presumably due to apoptotic degradation of nucleic DNAs. Identification of these dead cells from live cells can improved by morphometric features.

### *The needs of ImageStreamX on yeast aging research*

First, the important role of cell morphology on asymmetric division of aging factors and yeast aging can be studied by the requested *ImageStreamX* at a depth and scale that cannot be achieved by either conventional flow cytometry or confocal microscopy. The dividing cells in G2/M phases are a small fraction of cell population even in log-phased cells, and they are difficult to quantify by the confocal microscopic method. ImageStreamX has been used to monitor asymmetric distribution of molecules during mitosis [84], and identify cells in G2/M phase effectively from a large population of cells [84-86]. Morphometric measures of yeast cells by ImageStreamX have been found to correlate very well with nuclear staining patterns and cell cycles [86]. Budding morphology can be effectively studied at the population level with ImageStreamX , such as bud length, length ratio of bud and mother cells, ratio of width versus length for both buds and total cells (Figure 2) [85, 86]. The role of cell morphology in the mitotic asymmetry is a recently discovered topic [61], and better understanding on this subject can be quickly gained by conducting statistical imaging studies in yeast cell populations. Using ImageStreamX, we can address this question by simultaneously monitoring cell morphology and asymmetric distribution of ROS and dysfunctional mitochondria between mother and daughter cells in the subpopulations of cells in G2/M phases.

It is worthwhile to mention that cell cycles can be synchronized in haploid yeast strains. However, we are not aware of any methods to synchronize cell cycles in wild isolates, which are mostly diploid with heterozygous mating types.

Second, age-structures of yeast cell populations can be effectively studied by ImageStreamX. The replicative age of yeast cells can be monitored by various fluorescence probes (Table 2). Study aging structure by confocal fluorescence miscopy is tedious and could not be done for a large number of cells [66]. Using ImageStreamX and image analysis, replicative age can be estimated by counting fluorescence spots for bud scars. In addition, it is unclear whether budded and unbudded yeast cells die in different dynamics during chronological aging. Using ImageStreamX, the fate of budded and unbudded cells can be tracked by combining morphometric and photometric features [87].

Third, many technical challenges in studying yeast aging can be effectively addressed by ImageStreamX. For example, yeast cells, especially natural isolates, often stick to each other, which can complicate the interpretation of conventional flow cytometry. With ImageSteamX, attached cells can be distinguished from and S-phased and G2-phased cells based on nuclear morphology. Abnormal morphology can also be monitored [85, 86, 88]. Many yeast strains show different levels of background staining by propidium iodide (PrI) during live/dead assays. ImageStreamX can distinguish the large PrI nuclear DNA staining from the more diffused PrI staining of RNA and mitochondrial DNA.

Overall, ImageStreamX can greatly advance our knowledge on the ***morphometric*** and ***population*** aspects of yeast aging, especially cell morphology variation, asymmetric partition of aging factors (such as various ROS) during mitosis, and age-structure of cell populations. These aspects of cellular aging cannot be studied by conventional flow cytometry, and is difficult to quantify at population levels by confocal microscopic methods.

### *The impact of ImageStreamX on yeast aging research*

Understanding distribution and heterogeneity is critical for us to understand dynamic behavior of cells as systems. Aging is clearly a dynamic process and is greatly influenced by nongenic factors: A homogenous population of cells with the same genotype will inevitably live to different ages. Population heterogeneity, such as age structure, cell cycles, and physiological states, can be influenced by genotypic variation, stochastic variation [89-92], network dynamics, and environmental cues and noises. Therefore, quantification of population heterogeneity in natural isolates and deletion strains of yeast can advance our knowledge on these interacting factors.

PI Qin recently proposed a mathematic model for cellular aging based on gene interaction networks and the reliability model of aging [93]. Cellular aging is an emergent property of these gene networks models, and stochastic noise is a key factor for this emergence. This work was presented at the CSHL meeting on Computational Cell Biology, and a manuscript is in preparation. This emergence model is consistent with fact that aging is a complex trait and are influenced by many genes. Quantified natural variations (differences between populations) can be used to infer gene interaction networks through genome wide association study or quantitative trait analyses. Quantified population heterogeneity (differences within populations) can be used to infer the effect of stochastic noises and environmental cues on the determination of steady states of cellular systems.

Currently, most studies on yeast aging focus on the average life span, a simple statistic that does not adequately describes the dynamics of aging process. This limitation is part due to the lack of effective methods to monitor aging characteristics in large yeast cell populations. With ImageSreamX, we can advance the current field of yeast aging research to a new level of systems biology.

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| **Table 2. Key fluorescent probes/detection methods available for the yeast aging project** | | | |
| Probes/Methods | Features | Excitation peak (laser)/Emission peak | References |
| Bright-field images | Cell shapes, bud orientation. | Non-fluorescent stains can be monitored through red, green, and blue channels. | ImageStreamX specification |
| Propidium iodide (PrI) | Membrane permeability, nucleic acid content | Ex 536nm (488nm laser), Em 617nm | [84, 94, 95] |
| TO-PRO 3 iodide | Membrane permeability, nucleic acid content | Ex 642nm (658nm laser), Em 661nm | [96] |
| 7-Aminoactinomycin D (7-AAD) | Membrane permeability, nucleic acid content | Ex ~540nm (488nm laser), Em 647nm | [97] |
| SYTOX AADvanced Dead cell stain | Membrane permeability, nucleic acid content | Ex ~540nm (488nm laser), Em 647nm (Also available in other colors) | Invitrogen |
| FUN1 | Vacuole function and viability | Ex ~480nm (488nm laser), Em: ~540nm (green in dead cells) and ~ 590nm (orange red in live cells) | Invitrogen |
| FM4-64 | Vacuole function and viability | Ex ~480nm (488nm laser), Em: ~740nm (broad peak down to 650nm) | [98, 99] |
| SYTOX Green | Nucleic acids in dead cells (cell cycle and viability) | Ex 504nm (488nm laser), Em 523nm | [100, 101] |
| Calcofluor White M2R | Bud scars (replicative age) | E365nm (405nm laser), Em 435nm | [41, 57, 66, 100, 102-104] |
| Solophenyl Flavine 7GFE 500 | Bud scars (replicative age) | Ex ~390nm(405nm laser), Em ~500nm (broad peak, blue-green) | [105]. |
| Pontamine Fast Scarlet 4B | Bud scars (replicative age) | Ex ~510nm(488nm laser), Em ~580nm (broad peak, yellow-red) | [105]. |
| 2’,7’-dichlorodihydro-fluorescein diacetate (H2DCF-DA) | Mainly H2O2 but there are disagreements. | Ex 490nm (488nm laser), Em 530nm (sharp peak). | [56, 96, 106, 107] |
| Dihydrorhodamine 123 (DHR) | Mainly H2O2 and mitochondrial potential | Ex 511nm (488nm laser) Em 534nm (green) | [55, 56, 95, 106] |
| Didydroethidium (DHE) | Mainly superoxide anion (DNA content) | Ex 510nm (488nm laser), Em 570nm with broad peak | [55, 56, 96, 106, 108, 109] |
| JC1 | Mitochondrial potential | Ex 488nm laser, Em 527/590nm | [110] |
| Nonyl acridine orange (NAO) | Cardiolipin levels (Mitochondrial mass) | Ex 490nm, Em 518nm | [111, 112] |
| DiSC3(5) | Mitochondrial potential | Ex 633nm laser, Em >680nm | [95, 113]. |
| DiOC6(3) | Mitochondrial potential | Ex 490nm (488nm laser), Em 505nm (similar dyes in other wavelengths also available) | [95, 114, 115]. |

### *Research plans for population dynamics study on yeast aging*

Our main objectives are: 1) Characterize the morphological distribution, intracellular distribution of ROS, and/or replicative age-structure in yeast cells populations from log-phase, diauxic shift, and stationary phase; 2) Characterize the morphological distribution, intracellular distribution of ROS, and replicative age-structure in yeast cells during chorological aging; 3) Compare the measured population dynamics in yeast natural isolates and deletion strains. By accomplishing these objectives, we will generate information with unprecedented details on the roles of morphology, mitotic asymmetry, ROS, and age-structure in yeast aging and natural variation of yeast life span. We will then be able to gain insights on the causal connections of these factors using statistical analysis and modeling.

The Qin group previously characterized RLS, CLS, and age-induced genomic instability for a collection of yeast natural isolates [3, 5, 7]. In addition, the Qin group is in the process of phenotyping over 60 yeast natural isolates with sequenced genomes. Half of these strains were purchased from the Sanger Center, and half of them were generously provided by Justin Fay at the Washington University at St. Louis.

We will also focus on yeast deletion mutants with extremely long and short life spans, and on mutants in well-studied pathways, such as TOR1 and SIR2. The Kaeberlein group measured RLS for 564 yeast deletion mutants [116, 117]. The Qin lab has purchased the yeast deletion library with the support of a NSF RUI grant. Parental strains and strains with average life spans will be used as controls.

Yeast deletion mutation with known effects on morphology will be selected from the *Saccharomyces cerevisiae* Morphological Database (SCMD, http://scmd.gi.k.u-tokyo.ac.jp/). SCMD provides a list of 501 morphological parameters in four groups: cell shapes, bud sizes, nucleus locations, and actin localizations [118]. Serendipitously, three of them are related to cellular aging. Cell shape and bud size are related to mitotic asymmetry, and actin dynamics are known to influence mitochondria function and ROS levels [115, 119]. These parameters were measured by microscopic approach, and only mean values were provided. Using linear regression, Miss Charita Montegomery, an undergraduate in my group, found that two morphological parameters, C125 and D203, are associated with replicative life span at a false discovery rate of 5%. C125 is the ratio of bud size to mother cell, and D203 measures the condensation of nucleus. Hence, we plan to focus on yeast deletion mutants with high, medium, and low values of C125 and D203.

Age structure can be measured by bud scar staining. Each cell division in yeast will leave a bud scar on cell wall [120]. Fluorescent intensity of bud scars can be used to estimate the replicative age of cells [65, 66, 70] (Table 2). Although bud scars can overlap, the chance of overlapping scars is very small in cells with low number of scars, especially when cells with more than 6 bud scars start to enter apoptosis [66].

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| Figure 2. Budding morphology and cell cycle can be effectively quantified by ImageStreamX. Dashed cross-lines the on the left can be measured, and their ratios indicate morphological changes. Morphological ratios correlate very well with nuclear patterns during cell divisions. Adopted from [85, 86, 88, 121]. |

In addition to morphology and age structure, population heterogeneity can be further monitored by a substantial number of commercially available fluorescent probes for various ROS, mitochondria function, vacuole, nucleus DNA, and membrane integrity (Table 2). Yeast cell cycle can be classified into no bud (G1), budded cell with a single nucleus (S-phase), or budded cells with two nuclei (G2) by morphology and DNA staining (Figure 2) [85, 86, 88, 121]. We also have the option to measure mitochondria mass and potential by JC-1, vacuole changes by FM4-64, capase activity by FITC VAD-fmk [115, 122], and actin cytoskeleton by rhodamien phalloidin that is known to influence mitochondria function and ROS levels [115].

We are especially interested in working out a protocol to monitor morphology, bud scars, ROS levels, and viability simultaneously. We plan to stain cells with calcofluor white M2R for bud scars (excited by 405nm laser and monitored at 430-505nm), TO-PRO3 for dead cells (excited by 642nm laser and monitored at 640-745nm), and a choice of probe to detect ROS levels such as DHE (excited by 488nm laser and monitored at 560-595 nm) (see Table 2). A full-colored bright field image will also be recorded. To address the potential spillover of fluorescence to adjacent channels, control images will be generated from unstained samples (negative controls) and single-stained samples (positive controls), and a compensation matrix will be calculated using the IDEAS software to automatically correct for spectral overlap.

We will first perform single-dye experiments to efficiently characterize a large number of strains, and group strains into several phenotypic categories. We will then study representative strains in details by multi-dye experiments.

Yeast chronological life span will be measure in both water and depleted media for comparison. Hydrogen peroxide and menadione will be used to challenge yeast cultures. Yeast culture will also treated with calorie restriction and rapamycin to illustrate their effects on life span, ROS, and age-structures. It is worthwhile to emphasize that for yeast cells in nutrient limiting conditions, they do not die due to starvation per se, because there are substantial amount of reserve glycogen and trehalose remained even when most of the cells have died [123].

The Qin lab previously demonstrated a statistical association between genomic instability and yeast life span variation in a collection of natural isolates of yeast, which suggests a potential link between ROS and genomic instability. This link can be tested by double-stain with ROS probes and DNA probes. A “mask” of the DNA probes will be created to pick the ROS signals co-localized with the DNA probes. ‘Similarity score’ of pixels intensities between the images of ROS images and DNA images (such as TO-PRO3, Hoechst, DAPI, or DRAQ 5) will be calculated for each cell. The similarity score is a log-transformed Pearson’s correlation coefficient available in the IDEAS software [124] (see also IDEAS user manual provided by AMNIS). We will then study the causal association of the similarity scores distribution, life span distribution, and genomic instability in the collection of natural isolates in the Qin lab. We understand that this experiment is technically challenging and will likely required advanced image analysis. The data analysis software (IDEAS) has numerous parameters that are employed to discriminate cells based on size, shape, texture, location and co-location of probes. In addition, the software has a user-guided analysis capability that automatically selects the features that best differentiate cells that the user has chosen. This routine leverages the innate ability of humans to distinguish cells based on their staining patterns, and that of the software to calculate, rank and select quantitative features that best discriminate the chosen cells, thus making image analysis with this platform accessible for beginning users. Moreover, fluorescence images can be export to TIFF files and analyzed by customerized scripts using Bioconductor EBImage package [125], if necessary.

## *Plans to address potential challenges*

We understand that membrane integrity and mitochondrial potential are different proxies of “viability” [100], as compared to the colony-forming units (CFUs). We found that yeast cells killed by heat-shock can lose colony forming ability without losing membrane integrity, and many of them are unstained by PrI. We also found that life span determined by membrane integrity are often longer than life span determined by CFUs, suggesting that many mitotically defective cells still maintain membrane integrity. To address these concerns, we will measure CFUs in parallel with the ImageStreamX measurements. In addition, CLS of many strains under study have been characterized using the CFU method [3, 5]. We are in the process of performing CFU based viability assays for more strains.

We expect that the spot counts from bud scar staining using ImageStreamX is an approximate proxy of the actual number of bud scars, due to ImageStreamX’s high-throughput nature. To address this systematic bias, we will compare bud scar distributions from both ImageStreamX and confocal microscope, and generate calibration curves. We understand that it is tedious to quantify bud scars with confocal microscope, and we only conduct calibration for a few strains. These calibration curves are likely to be approximately linear in the low range of bud scars. We would also like to mention that this kind of ‘consistent’ systematic bias usually would not alter conclusions drawn from regression based statistical analysis.

There is a chance that our primary fluorophore choices for multi-color experiment may not work. Given the wide range of excitation and emission peaks of probes (Table 2), it is reasonable alternative combinations of double and triple stains are possible. Furthermore, some of the probes have been shown to work simultaneously. For example, H2DCF-DA, DHE, and TO-PRO3 were used to monitor H2O2, O2\*-, and viability simultaneously [96]. H2DCF-DA and PrI were used simultaneously to monitor apoptotic features [126, 127]. Double stain of live yeast cells with FM4-64 (red) to monitor vacuole and with Hoechst (blue) to monitor DNA content can be done by established methods [98]. Hoechst and PrI can be used to monitor both live and dead cells [128]. Invitrogen provides a free iPad app to simulate the multiplexing of fluorophores. We have been using this app to guide experimental designs of flow cytometry.

We are aware that PrI can stain nucleic DNA, RNA, and mitochondrial DNA. Its intensity can also be influenced by DNA condensation [84, 129], and its emission spectrum is too broad for multi dye studies. We plan to use PrI mostly in single-dye experiments and in large scale surveys, due to its low cost and operational simplicity.

## *Results from Qin’s prior NSF support*

### Dr Qin’s current research on yeast aging is supported by NSF RUI award #1022294, “Testing a network hypothesis of cellular aging in *Saccharomyces cerevisiae*” (2010-2012). The main goal of this RUI grant is to test a hypothesis that cellular aging is an emergent property of gene networks. This support has enabled Dr. Qin to engage 14 undergraduate researchers (all are African American females) in the research projects in his lab. This support has resulted in 1 publication [7] and 3 manuscripts in preparation (Asterisks indicate undergraduate co-authors): (1) Parnell, L\*, E Jackson\*, M Parker\*, J Rodrigues, H Qin, “Natural isolates of *Saccharomyces cerevisiae* show a diverse pattern of genomic instability in response to hydrogen peroxide”. (2) Story A\*, C Montgomery\*, C White\*, H Qin, “Robustness and cellular aging in *Saccharomyces cerevisiae*.” (3) Qin, H. “Emergence of cellular aging from gene network models”. (This paper developed a mathematic framework on yeast aging and showed that cellular aging is an emergent property of gene networks.)

Dr. Qin was supported by a NSF CCLI grant during 2009 (award #0837075), “Computing in life sciences through hands-on experience and case studies at Tuskegee University”. This grant enabled Dr. Qin to develop many course materials to teach computing to biology students. These materials are available at <http://www.bioiformatics.org/ctls>. This grant led to 1 publication based on Qin’s pedagogical research on teaching computational thinking to biology students [130].

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## B.2 Function ofglycosphingolipids in cell proliferation and apoptosis. (Maloney)

Glycosphingolipid expression varies greatly from one cell type to another, yet very little information is available on the role of the various glycolipids in functions of different cell types. Research in the Maloney lab focuses on defining the function for globotriaosyl ceramide (Gb3), a glycosphingolipid. Gb3, also known as CD77, is a marker for germinal center stage B cells [131]. Gb3 can also be found in many other cells with high proliferation rate, such as epithelium and endothelium cells [132, 133]. Known or suspected roles for Gb3 include functions in CD19-mediated cell adhesion, interferon-alpha signal transduction, MHC class II-mediated antigen presentation and apoptosis pathways [9, 11-16, 131]. Because the Burkitt lymphoma (BL) cell line, Daudi, is similar to germinal center stage B-cells in their expression of Gb3, CD19, and MHC class II molecules, it has been used as an *in vitro* model for the Gb3-expressing germinal center stage of B cell development found within lymphoid tissue. VT500 is a Gb3-deficient mutant cell line that was derived from the Daudi line, and it has been used as a null control to study the function of Gb3 [12, 15]. A region of the N-terminal extracellular domain of CD19 is similar to the Shiga toxin/verotoxin B-subunit [9]. The cell surface receptor of Shigatoxin B subunit is Gb3 [133-135]. Therefore, the B subunit was thought to mimic CD19 function and has been used to study the interaction of CD19 and Gb3 [9].

In Maloney lab’s previous investigations, flow cytometry and/or western blot analysis indicated that surface levels of CD19 correlated with the levels of Gb3 expression in BL cell populations and subclones, and that whole cell expression of MHC class II molecules increased with Gb3 expression while surface MHC expression remained constant [9, 11, 12, 14, 15].

Several important questions with regards to the function of Gb3 can be studied in greater details by ImageStreamX. These questions include the intracellular trafficking of the Gb3/CD19 complex and their differences between proliferating and apoptotic cells. It was recently shown that Shiga toxins/verotoxins undergo retrograde transport following binding of cell surface Gb3, moving back through the Golgi and ER and accumulating in the perinuclear space [133]. This suggests that CD19, which also interacts with Gb3, may undergo similar intracellular trafficking dynamics. Therefore, we can gain insights on the function of Gb3 by comparing intracellular locations of Gb3, CD19 and HLA-D MHC class II proteins in both Gb3-expressing cells (Daudi) and Gb3 deficient mutant cells (VT500). Both Daudi and VT500 cells will be treated with combinations of Shiga/verotoxin, anti-CD19 antibody, and/or anti-HLA-D antibody. ImageStreamX will provide us with information regarding the subcellular location of CD19, Gb3 and potential Gb3-associated proteins in cell populations. This will assist us in determining the roles of Gb3 in signaling pathways and will allow us to compare protein trafficking in typical Gb3-expressing BL cells and Gb3-deficient mutants.

We have also found that susceptibility of BL cells to apoptosis inducers increases with Gb3 expression. We plan to use ImageStreamX to monitor apoptosis induction [136, 137] in the Daudi and VT500 cells following treatment with camptothecin, serum deprivation and other apoptosis inducers. ImageStreamX is especially suited for studying apoptotic features because of its combined morphometric and photometric capability. Morphological features of apoptotic cells include nuclear condensation, nuclear fragmentation, membrane blebbing, cell shrinking or swelling. Photometric features of apoptotic cells can be measured by terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay, the Annexin V assay for surface phosphatidylserine exposure, and fluorogenic caspase substrates to detect caspase activity. Monoclonal antibodies against Gb3 and CD19, and verotoxin will be conjugated with phycoerythrin (PE) or fluorescein (FITC) [15, 138]. Monoclonal antibody to Gb3 and CD19 are both commercially available. We plan to use DRAQ5 (Excitation with 658nm laser and detection at 660-740nm channel) as a nuclear dye because it will give us the option of using PE (detection at 560-595nm channel) and FITC (detection at 480-560nm channel). In addition, DRAQ5 can stains both live and dead cells [136]. Other choices of nuclear dyes, such as 7-AAD, have also been successfully used to study apoptotic cells by ImageStreamX and will also be considered [137]..

The Maloney group has 11 publications from studying Gb3-related topics and five undergraduate researchers have made sufficient intellectual contribution to earn co-authorships [8-18].

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## B.3 Dynamic behavior of androgen receptor (AR) in cell proliferation. (Jackson)

The oveall goals of this research is to understand the basic mechanism of cell proliferation. The Jackson lab particularly focuses on androgen depedent cell proliferation, and uses chemical invervation to study its mechanism. One chemical compound, dibenzoylmethane (DBM) (Figure 2) that is a natural constituent of licorice, is has been used extensively by the Jackson lab. The Jackson lab was one of the first to show that DBM can suppress the expression of androgen receptors and inhibit cell proliferation in androgen-responsive cell lines, such as LNCaP [22, 23]. DBM induced pronounced changes in LNCaP cells, causing an accumulation of cells in the G1 phase of the cell cycle as seen by flow cytometry [22] and altered key proteins as seen by proteomics [21]. The *in vitro* anti-proliferation effect of DBM been verified in transgenic mouse models by others [139].

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| **Figure 2.** Small molecule dibenzoylmethane (DBM) is a β-diketone compound, structurally related to dietary curcumin. Through GC/MS studies, the Jackson laboratory has confirmed DBM to be a constituent of Chinese licorice root *Glycyrrhiza glabra.* (manuscript submitted, 2011) |

Using protein 2D gel and mass-spec analysis [21], it was found out that DBM up-regulate phase 2 hepatic detoxification enzymes, valosin-containing protein, glucose-regulated protein 78, phosphoenolpyruvate carboxykinase (PEPCK), HSP70, HSP60, UDP-glucose dehydrogenase, Neuro-d4 (rat) homolog, mannose-6 phosphate receptor binding protein (Tip 47), and actin. Down-regulated proteins include heterogeneous nuclear ribonucleoprotein k (hnRNP K), FK506-binding protein 4 (p59), and creatine kinase B (CKB). These findings suggest farily intricated pathways underlying the anti-proliferation effect of DBM and its effect on androgen receptor expression.

Our objective in this proposal is to study the mechanism of the anti-proliferation effect of DBM and test the connection between DMB and the AR signaling pathway. We have hypothesized that this mechanism is through a non-competitive antagonism, likely via allosteric effects on AR and/or its co-activation partners. We will identify and model the allosteric AR-binding sites with specificity for DBM. Site directed mutagenesis will then be used to make alternative DBM-responsive AR mutants. This will allow for better understanding of the mechanism by which DBM affects AR binding and specificity. Using transiently transfected AR null prostate cancer cell with native and mutant eGFP-AR will permit better understanding of the dynamics of AR signaling in response DBM. The ImagestreamX cytometer will be used to address the following research questions: Does DBM bind to the AR or interfere with the receptor coactivators or the androgen response binding element? Does this DBM/AR interaction affect AR function, signaling and cell cycle regulation? As in other projects, ImageStreamX will be crucial in monitoring the intracellular trafficking of AR, such as nuclear translocation, in different cell cycle states, due to its unique combination of morphometric and photometric measures. As with other projects, DRAQ5 can be used to label nuclear DNA. Various concentration of DBM will be dosed to cells. The dose-dependent effects of DBM on cell populations will be evaluated by the proposed ImageStreamX.

The Jackson group currently has 4 peer-reviewed publications with 3 more manuscripts in preparation. She has engaged 35 Spelman students in her research, with an established track record of funding. Dr. Jackson has had over 15 years of training and experimentation in flow cytometry (3 other publications in flow cytometry, not related to current research).

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## B.4, Population dynamics of bacterial cells in responses to metal ions stress (Ibeansui, Qin)

Bacterial cells display characteristics of multicellular behavior, such as programmed cell death, cell differentiation, quorum sensing, and inter-cellular communication [[Hanna Engelberg-Kulka, 2006 plosgenetics]]. These multicellular-like behaviors in turn indicate heterogeneity in bacterial cell populations.

Apopotic characteristics, such as cell shrinkage, DNA condensation, and DNA degradation, was reported in Streptococcus pneumoniae cells, when they are induced by a human milk complex of alpha-lactalbumin and oleic acid [140]. *S. pneumoniae* cells rapidly lose membrane potential, in similar way to the loss of mitochondrial potential during eukaryotic apoptosis.

Metal ions are often essential for cell functions, but many of them are also environmental stress factors [[Iron and metal regulation in bacteria, Klaus Hantke]]. In *Escherichia coli*, the Fe2+ regulator Fur is regulated by OxyR and SoxRS, transcription factors activated by hydrogen peroxide and superoxide, respectively. Fur proteins also contain binding sites for Zn2+, and these sites can be occupied by other divalent cations. Similar connection between metal ion regulator and oxidative-stress response regulators are also found in *Bacillus subtlis*, *Streptomyces reticuli, Mycobacterium marinum,* and many other bacteria.

The Ibeanusi lab has intensively studied the effect of metal ions on bacterial community and population. The Ibeanusi lab found that, at a Savannah River Site, Aiken South Carolina, a coal pile run off led to the increase of metals including As, Cr, Cu, Cd, Fe, Hg, Pb, Se, and Zn in waters. The Ibeanusi lab found that a strain of *B. cereus* can detoxify these metal-contaminated waters, and has been studying the molecular and cellular mechanisms of this process. Preliminary study shows that metal-containing water induces unique protein expression in a strain of *Bacillus cereus*, which includes an enzymatic reductase. Given the known conserved genetic connection between metal and oxidative stress regulations in bacteria and yeast, we suspect that oxidative stress pathway is involved in the metal ion processing pathways in this strain of *B. cereus*.The Ibeanusi lab found that bacteria cells of mixed species generally survive much better in metal-containing water than do pure cultures of single strains, suggesting species interaction are involved. Furthermore, the Ibeanusi lab observed that some bacterial cells can undergo dramatic shrinkage in the presence of metal ions, suggesting apoptosis-like behavior for a sub-population of cells. Based on these findings, we propose to monitor the dynamics of ROS, membrane potential, viability, and intracellular metal ions to gain in-depth understanding of the biochemical and physiological changes of cell populations in responses to metal ions.

ImageStreamX will be used to monitor cell morphological changes, such as shrinkage, intracellular ROS levels and membrane potential changes, and membrane permeability, using probes described in section B.1. Technique report showed that length distribution of E. coli can be measured by ImageStreamX [141]. Many fluorescence probes can be used to monitor dehydrogenase activities [142]. Viability can be inferred based on membrane permeability and membrane potentials [142]. Intracellular metal ions can be monitor by a series of molecular probes available from Invitrogen, such as Calcium green-5N, Fluo-5N, Newport green DCF, Phen green FL, and calcein. All of these fluorescent probes have various levels of cross-reaction with different metal ions. Therefore, we will first conduct screen experiments to identify the proper probes for various metal ions. Tentatively, we plan to focus on Fe2+, Cu2+, Cd2+, and Pb2+ ions, based on the specifications provided by the vendor.

The Ibeanusi lab has recently initiated a project to study the aquatic microbe diversity and water quality along the Chattahoochee river. Concentrations of various metal ions will be measured in water samples collected at sites in six counties. In the proposed project, water samples from Chattahoochee River will be collected at different seasons of the year to monitor the bioavailability of metals. Conductivity, pH, temperature, cations, anions and dissolved oxygen will be analyzed using a Horiba Water Quality Checker (Sunbelt Scientific, Inc). The redox will be measured using a meter with a platinum-KCl electrode (Corning Inc.). Nutrient availability will be measured as total nitrates, and orthophosphates using Ion Chromatograph. Metals will be analyzed by digesting the samples using Microwave Digestion System (CEM Incorporation) and then measuring digested samples using Inductively Coupled Plasma (Plasma 400, Perkin Elmer).

The importance of ImageStreamX in aquatic microbial research can also be seen in NSF **MRI award #0923068** for an ImageStream **given to the** Scripps Institute of Oceanography. Dr. Mark Hildebrand, the PI of Scripps MRI award, generously shared their experience of using ImageStreamX with us. We will use ImageStreamX to record microbes in waters samples collected along the Chattahoochee river. Many microbes, such as unicellular phytoplankton, cynobacteria, can be monitored by its autofluorescence. Gram-positive cells can be monitored by wheat germ agglutinin (WGA) conjugated with a fluorescent dye [143].

Overall, this project will advance our understanding of metal ion regulations in microbial populations and microbial community interactions in environment [17] [144] [142, 145]. The Ibeanusi group has 6 publications [24-29] and 1 patent on bioremediation topics, and have extensive experience of mentoring undergraduate in research in environmental sciences.

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## B.5, Student independent studies and course projects (see also D.1 and D.2)

Both Biology Department and Environmental Science and Studies Program at Spelman offer research courses (BIO487 and ES435). Students in these courses are expected to conduct hypothesis-driven research projects with a faculty mentor, and are encouraged to propose their own research projects. One student, Miss Morgan Maite, recently proposed to study cellular aging in *E. coli* using (conventional) flow cytometer. Her idea was supported with enthusiasm by PI Qin. The requested ImageStreamX will give Spelman students an opportunity to propose their independent research projects using a state-of-the-art technology. Please see section D.1 and D.2 for more discussion on student research and course projects.

# C. Description of the Research Instrumentation and Needs

## C.1 The Instrument

The requested instrument configuration is attached as a supplementary document. The basic model of ImageStreamX (AMINS Item # 100200) includes six imaging channels, 40X magnification, single color brightfield, a 488nm excitation laser, a 785nm darkfield laser, and a single-user license of the IDEAS software. It also covers the installation process, support training of two persons at AMINS, and one-year parts and labor warranty. Because of our research needs, we request a 405nm violet excitation laser (for calcoluor white M2B, DAPI, and Hoechst 33258), a 658nm red excitation laser (for DRAQ5 and TO-PRO3), a full color bight field, an extended depth field and a 60X magnificant upgrade. The upgrade of depth field and magnification would greatly improve the accuracy of bud scar counting of yeast cells and improve resolution for bacterial cells. The CCD camera in ImageStreamX is able to capture 12-bit image with 0.5um per pixel. A 60X magnification upgrade will give a resolution of 8.5nm per pixel. The additional lasers will give us more flexibility to use multiple fluorophores. An autosampler is requested to improve the handling efficiency especially for the yeast aging project where a large number of yeast strains are assayed.

We also requested a data analysis workstation computer with 1.5TB RAID and dual 24 inch monitors for data storage and off-line data analysis (as recommended by AMNIS). Customized masks and boolean logic to selection area in images are available in IDEAS. Images generated by ImageStreamX can also be converted to TIFF formats for analysis by other tools, such as R, matlab, or Python.

## C.2 The Needs

The scientific needs of ImageStreamX are due to its unique technical capability. ImageStreamX is essentially a combination of a flow cytometer and a fluorescence microscope [87, 124, 136, 137, 146-152]. As such, it can record up to 12 fluorescent images per cell, and efficiently describe the statistical distributions of these morphometric and photometric measures in a large number of cells. This statistical imaging capability can describe cell population dynamics in a depth that cannot be matched by flow cytometer and in a scale that cannot be matched by fluorescence microscope. Understanding distributions and dynamics is a key distinction of systems biology, in contrast to the focus on norms in classical biology [153]. Population distribution is especially important for the understanding of biological aging. Biological aging is defined and quantified by distributions of life history-traits over age (i.e. time) in populations [40, 76, 154-157]. Different types of distribution of aging-related traits suggest different underlying mechanisms, and is a distinction between biological and non-biological aging [77, 93, 158]. With the requested ImageStreamX, we can quantify cellular aging process in unprecedented depth and scale, and propel the current research field of yeast aging to a new level. Moreover, we will gain new insights on glycosphingolipids, cell proliferation, and environmental microbiology in other three projects on cell population dynamics.

The institutional needs of ImageStreamX are can be found on our unique institutional setting and the lack of equivalent instrument in proximity and. To the best of our knowledge, AMNIS is the only vendor that offers a ready-to-go commercial version of image flow cytometer. The closest ImageStreamX to us is 158 miles away at the Medical College of Georgia in Augusta, Georgia. We are not aware of another ImageStreamX in the Atlanta area. This gives the Spelman College, a black women’s college, an opportunity be a regional leader in statistical imaging research (see also section D.3).

# D. Impact on Research and Training Infrastructure

The impact can be found on research training of minority students, curriculum transformation, enhanced faculty research and collaboration, and enhanced research capacity at a historically black college for women.

## D.1 Research training of minority students

## The PI and coPIs, as with many faculty at Spelman College, fully embrace the notion that learning by investigation is the most effective way of learning. The PI and coPIs have demonstrated records of engaging undergraduates in research (summarized in Table 1). Each year, about 8-9 Spelman students will be trained to use the requested ImageStreamX to conduct experiments for the 4 proposed projects (Table 1). These Spelman students will gain ownerships to a unique body of data and knowledge, because of the capability of ImageStreamX and its relatively new status in research. The high-throughput nature of ImageStreamX also means that research projects will be data-rich, and students will receive state-of-the-art training in the computational and statistical aspects of modern biology research. This kind of interdisciplinary training and the combination of experimental and computing experience will greatly enhance student preparation for graduate school. Moreover, we will encourage our students to present their findings at various national and international meetings. These meetings will enable our students to present their accomplishments on statistical imaging and cell population studies, and to learn more about graduate schools and career opportunities in science.

In addition, both Biology Department and Environmental Science and Studies Program offer research courses (BIO487 and ES435), and encourage students to work as research assistants. The Spelman Biology department is also preparing for a research minor (A HHMI proposal was submitted recently). ES435 is a required course for a minor in Environmental Science and is often fulfilled by independent research projects. The requested state-of-the-art ImageStreamX would bring a sense of excitement to students and would a highlight of our recruitment for the research students.

## D.2 Curriculum transformation

As a liberal arts college, education is our first and foremost priority. There is currently a campus-wide drive to increase student participation in research under the Spelman MILE program, a program designed for the Quality Enhancement Program (QEP) at the Southern Association of College and Schools (SACS). The requested instrument and proposed research projects will enhance the investigative, quantitative and/or computational aspects of curricula in Biology, Chemistry, Environmental Science and Stuides, and Computer Science (Table 3).

The Spelman Biology department is constantly revising its curriculum to provide students with the best available learning experience. The requested ImageStreamX can be used by small groups of undergraduates in BIO328, BIO320, and BIO233 (see Table 3), and give hands-on experiences to dozens of students.

The Biochemistry program at Spelman is certified by the American Chemical Society (ACS). The goal of the CHE 313 laboratory course is to provide a research-based learning environment, and seeks to develop the student´s skills for critical analysis of research results and scientific literature, provide guidance in technical writing and engage students in learning laboratory skills focused on collecting and interpreting data from a real science problem. The image flow cytometer will be used to study cell cycle regulation and apoptotic pathways of HeLa cells dosed with various chemical reagents.

The Program ofEnvironmental Science and Studies actively develops multi-disciplinary courses to prepare students to become leaders in a diverse workforce that are competent in addressing major environmental issues. ImageStreamX is extremely suitable for environmental microbial monitoring, and can be potentially incorporated into several courses, including Ecology (ES/BIO 255), Environmental Biology (ES/BIO 314), and Introduction to Environmental Science (ES211). We will first pilot the usage of ImageStreamX with research students, and then decide which ES courses are best suited to adopt ImageStreamX in practice.

In addition, the data-rich nature of image flow cytometer will strengthen the interdisciplinary and quantitative aspect of our curriculum. The data generated by ImageStreamX can also be used by BIO120 students in experimental design, BIO320 for computational biology project, and CIS115 for bioinformatics projects (see Table 3).

#### Table 3. Courses impacted.

|  |  |  |  |
| --- | --- | --- | --- |
| Courses | UG usages of ImageStreamX | ImageStreamX components | Instructors, enrollment per semester |
| BIO328 Immunology | Direct use in groups | Project on B cell development | Maloney,  ~25 students in 5 groups |
| BIO233 Microbiology | Direct use in groups | Projects on microbial diversity, effect of oxidants and antibiotics. | Hong Qin,  ~ 20 students in 4 groups |
| CHE 313L Biochemistry Laboratory | Direct use in groups | Projects on cell cycle regulation, apoptotic pathways, and their influence by various chemical compounds. | Kimberly Jackson,  ~10 students in 5 groups |
| BIO320 Genomic, Proteomics, and Bioinformatics | Data analysis | Projects on cell population, morphology, aging, growth fitness, and expression profiles analysis will be introduced. | Hong Qin, ~ 10 students |
| CIS115 Introduction to Computing and Informatics | Data analysis and informatics | Coding projects on image analysis, informatics on multi-dimensional data, and classification of cell morphologies. | Alfred Watkins, Hong Qin, ~20 students |
| Bio120 Cell Biology | Data usage | In-class experimental design on cell cycle and cell population studies. | Maloney, ~ 48 students |

UG: undergraduates. BIO, CIS,CHE indicate courses in biology, computer science, chemistry, and mathematics. All of the courses are offer once per academic year.

## D.3 Enhanced faculty research and research capacity at a HBCU institution

This project will invigorate research programs and boost research capacity at a historically black college for women. Spelman College is a private, independent, historically black college for women with 130 years of history. Over 95% of Spelman students are female African-Americans. Spelman is the only historically black college to be included in the U.S. News and World Report’s list of top 75 “Best Liberal Arts College – Undergraduate” for many years. Our strategic plan for 2015, termed Spelman MILE, aims to ensure key competencies: critical thinking, effective communication, quantitative reasoning, and digital literacy, that students need for personal success. The Spelman MILE recognizes that undergraduate research/internship is critical for students to acquire these key competencies.

The requested ImageStreamX is a fairly new technology, and it will enable participating Spelman faculty to become the first to apply this state-of-the-art statistical image technology to study cell population dynamics in each of their own fields. ImageStreamX will especially advance the scope of questions that Dr Qin’s group can address on yeast aging. Outcomes from this project will enable Qin to conduct genome wide association study to identify loci associated with changes of life span variations, morphology, ROS levels, age-structure in natural isolates of yeast. Currently, the Qin lab is surveying life span and tolerance to oxidants of many yeast strains and natural isolates under the support of an NSF RUI grant, and is preparing for another NSF research proposal. Overall, ImageStreamX will greatly improve the depth of investigations on cellular aging, cell development and proliferation, and environmental microbiology. The data-rich and quantitative aspect of image flow cytometer will foster collaboration across disciplines, and will greatly improve the competitiveness of our future proposals.

Overall, the requested ImageStreamX would propel the statistical imaging research capacity of Spelman College to the top of the nation. Spelman College would be the second liberal arts college and the first HBCU to have an ImageStreamX (The other is Nevada State College). This state-of-art research capacity will help Spelman College with faculty recruitment and retention, and in turn, ensure the best available learning experiences to Spelman students.

# E. Management Plan

## E.1 Operation and maintenance

PI Qin will oversee the operation and maintenance of requested ImageStreamX, and coPI Jackson and Maloney will serve as mentors to Qin. Qin has 2.5 years of experiences of using flow cytomter, and Jackson and Maloney both have more than ten years of experiences. The likely locations of ImageStreamX and workstation, Room 260 and 258, are just across hallway from Dr. Qin’s laboratory at Room 246.

Qin will be a primary user, but access to the instrumentation for all researchers and their students will be arranged by mutual agreement. A sign-up sheet will be used to reserve and schedule instrument run time. Reservation is especially important for planning experiments during summer when PI and co-PIs will engage all of their effort on research. A log of maintenance and experimental runs will be used to ensure that proper procedures have been followed. We request two additional years of service contract, especially giving the number of undergraduate users.

## E.2 Training of faculty and undergraduate users, and usage of the instrument

PI Qin and co-PI Jackson will be responsible to train other faculty users and students users. A three-day on-site training session of ImageStreamX and IDEAS software is budgeted for the training of Qin, Jackson, and a technician.

We believe that direction operation of ImageStreamX will give valuable hands-on learning experiences to our students and give them a sense of accomplishment and confidence. Cares will certainly be taken to ensure desirable outcomes. Student users of ImageStreamX will be classified to two groups: Frequent users and infrequent users. Frequent undergraduate users include student researchers in the proposed four research projects and those working on independent study projects using ImageStreamX. These students are required to be trained twice and to pass a practical test (given by PI Qin) before they are certified to use ImageStreamX independently. These students will also learn to use IDEAS to analyze cell populations. Training sessions led by PI Qin will be video-recorded and posted on Qin’s lab website at http://sunrays.spelman.edu/hqin. (Students will not appear in the posted video). These certified student users can work as TAs for the infrequent student users.

Infrequent student users include the one-time users from most course projects. These students are expected to learn the basic principle of ImageStreamX during lectures, to watch training videos before experiments, and to pass a written quiz on the basic principle and usage of ImageStreamX. These infrequent users must operate ImageStreamX following the instructions of a trained faculty or TA. A faculty or TA will also lead these infrequent users to do data analysis using IDEAS using the requested data workstation. A Facebook group will be created as a discussion forum for Spelman student users of ImageStreamX.

There are likely some unforeseen challenges for training and managing undergraduate users for a sophisticated instrument like ImageStreamX, and it is likely that we will have to frequently revise our training plans. We argue that the operational risks are out-weighted by the valuable learning experiences of Spelman students gained through direct operation of the requested instrument. For this reason, we request two additional years of service contract to ensure successful outcomes of this project and to protect the investment of taxpayers.

## E.3 Institutional commitment and support, and long-term operation

The requested ImageStream will greatly enhance the student research experiences and interdisciplinary training, and fits into the college strategic plan of Spelman MILE. The requested instrument will also give Spelman students an opportunity to be leaders of their peers on cutting-edge technologies and research, and thus contributes the college mission of leadership training. The research projects of Qin, Jackson, Maloney, and Ibeanusi have all received considerable prior supports from the college, and this proposal is one of the fruitions of those supports. (See support letter of Dr. Carmen Sidbury, associate provost for research).

The Albro-Falconer-Manley Science Center, the location of the requested ImageStreamX, is a $33 million building with over 140,000 square feet for teaching and researching. The research core facility in the Science building hosts a confocal fluorescence microscopy (Zeiss observer 2.1) and a BD FACSCalibur flow cytomer that can be used to support the proposed research projects. Dr Qin’s laboratory has a Bioscreen C instrument that can measure growth curves of yeast strains in high-throughput fashion. The core facility, biology and chemistry departments also have ultra centrifuges, deep freezers, tissue culture incubators, hoods, gel documentation system, a real-time PCR machine, and various other experimental equipments.

Support for supplies for running experiments on ImageStreamsX will come from research grants (for faculty research projects), department support (for student research projects and course projects), and various college-level programs (often in the form of pilot grants).

The cost for long-term maintenance of the request ImageStreamX will be defrayed through the participating groups and future research grants (one of the outcomes of this proposal) and the research core facility. PI and co-PIs have all received funding from various federal agencies. The requested ImageStreamX can increase the competitiveness of our future proposals.

## E.4 Assessment and dissemination

### Instrumental usage will be monitored by logs, the number of experiments, and the number of running hours. Research impact will be assessed by the number of peer-reviewed publication and future grant proposals. Increased faculty collaboration will be measured by the number of co-authorships and co-PIs. Undergraduate participation will be assessed by the number of student researchers, meeting presentations, and co-authorships. Information on long-term impact on undergraduate career path will be gathered by the proposed Facebook group for student users.

Dr. Robin Herlands, who has incorporated ImageStreamX into teaching at the Nevada State College, will collaborate with us on developing teaching materials. As the only HBCU and the second liberal arts college to use ImageStreamX in undergraduate based research, we will share our experiences of integrating research into curricula through meeting presentations, websites, a publication in teaching journals.

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